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BELGRADE - SERBIA

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ORGANIZER

Institute for Animal Husbandry
Autoput 16, P. Box. 23, 11080, Belgrade-Zemun, Serbia
Tel: +381 11 2691 611;
+381 11 2670 121;
+381 11 2670 541;
Fax: + 381 11 2670 164;
biotechnology.izs@gmail.com
www.istocar.bg.ac.rs

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Proceedings of the
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THE PRESENT AND FUTURE LIVESTOCK PRODUCTION – CASE STUDY SERBIA

M. Lukić[1], Z. Pavlovski[1], Z. Škrbić[1], K. Umetsu[2], V. Petričević[1]

[1]Institute for Animal Husbandry, Autoput 16. P. Box 23, 11080 Belgrade-Zemun, Serbia
[2]Graduate School of Animal and Food Hygiene, Obihiro University of Agriculture and Veterinary Medicine, Obihiro 080-8555, Japan
Corresponding author: miloslukic.izs@gmail.com
Invited paper

Abstract: Goal of this study is to summarize the present and future challenges facing livestock production worldwide, to point out some innovations and trends in livestock production in general and through the analysis. The material for analysis is Serbia, its state and outlook of agriculture and livestock production. Work briefly presents current situation in livestock production, main problems related to these issues, potential possibilities and steps for solving these problems.

Key words: livestock production, future development, Serbia

Introduction

At the beginning, few words about the purpose of our scientific job now and in future. What are we doing, and what is our main mission in the widest sense? It is, actually, production of food. Closer orientation is science, especially science in agriculture, or even closer animal science, and final vocation and title of our job usually depend on specific scientific fields which we are engaged in (nutritionist, geneticist...). In future, it seems that our research ideas and research teams must be broader rather than specialized in narrow field in which we mostly prefer to work. Why is it necessary? It is not (or not only) in order to provide funds for our research. Simply, we are facing the upcoming dramatic environmental and climatic changes and the water, food and energy deficiencies. Our research must be up-to-date, must provide answers to solving current or predicting future problems, open to new challenges and conquering new research fields (e.g. nutrigenomics). More and more, research in applied science generally, and in agriculture and food production particularly, must solve needs and problems, find solutions for wide and safe practical applications. That's why the present and future needs and problems in the production of food generally, especially in livestock production, must drive our job and research directions.

Goal of this study is very demanding, but valuable: to try to summarize the present and future challenges facing livestock production worldwide generally and through the analysis of situation in Serbia.

Agriculture and livestock production for future

What we expect from the future agriculture

First and foremost, they must feed the human population which, according to estimates, in the coming decades will exceed 9 billion people, more precise 9.3 billion by 2050 and 10.1 billion by the year 2100 (*Alexandratos and Bruinsma*, 2012). At the same time, agriculture need intensify in a sustainable way, meaning lower use of resources and less environmental pollution. Substantial prediction of future world agriculture production, as *Baulcombe (2010)* noted in *Science* issue dedicated to food security, can be that somehow the world must produce 50 to 100% more food than at present under environmental constraints that have not applied in the past. That "somehow", this radical transformation of agriculture, we must invent and apply worldwide in the next 40 years.

Seems that keyword of development in future, and not only of agriculture, is sustainability (*Godfray et al., 2010)*. Modern intensive livestock systems are more and more efficient in terms of high per-head productivity of meat, milk and eggs. On the other hand, intensification of livestock resulted in higher greenhouse gas emission, biodiversity loss, water pollution, pressure on arable land and animals themselves at farm, in country or at regional level. In some parts of world this problematic livestock development seriously and rapidly diminish natural resource base, and there is urgent need for better solutions (*Herrero et al., 2010*).

Drivers of livestock production

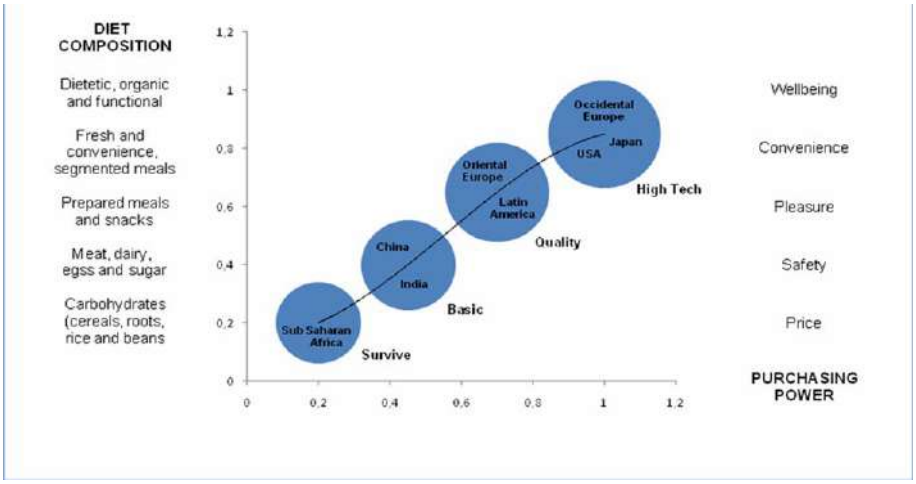
Generally, three key players direct the current and future development of livestock production: consumers, production systems (including state and government agricultural policies and legislation) and scientific community.

Maybe the most important are needs and requirements of the consumers, or how traders usually like to say „his royal majesty – buyer". Their most common requirements can be summarised in one sentence: Production of cheap, tasteful and high-quality food, ensuring the environment protection in the production process and respecting the principle of animal welfare!

Depending on the specific market, some of the requirements can be present, or all of them. The essence of differences in world food markets and

interconnections between food consumption/quality patterns and purchasing power of societies is shown in Graph 1 (adopted from *Roppa, 2012)*. These requirements are at the same time our final task.

Graph 1. Dependence between food consumption/quality patterns and purchasing power of societies (adopted from *Roppa, 2012)*



Development perspectives of any production sector depend on prospects for demand growth. From this point of view seems that livestock production has bright future. Estimated gains in real per capita income, rapid population growth and urbanisation in future, especially in the developing world, will generate increases in consumption of livestock product on that scale, which some authors anticipated and called "Livestock Revolution" (*Delgado et al*., *1999*; *FAO, 2011*). In coming decades, consumption (and production) of meat and milk will be increased primarily in South and Southeast Asia, Latin America and Africa.

Current state and especially future development perspective of livestock production in some country closely depend on policy and economic environment. Development of livestock production systems usually was firstly driven by profit, and secondly by states' and governments' agricultural policies and legislation frameworks. Generally, with regulations related to agriculture (including subsidies) governments in EU countries, USA and most developed countries effectively cope with strategic issues:

- Securing a stable supply of sufficient quantities of safe food for their population

- Facilitate agro-industry to modernize and develop, but also ensure reasonable living standards of its farmers
- Ensuring equal development of agriculture in all regions
- Support the development of living conditions in rural areas
- Improve the food quality
- Guaranteeing/monitoring food safety
- Ensure that natural resources are being preserved for future generations
- Improving animal health and welfare

And what is the position of the scientific community on future development of livestock production and on current and future jobs and tasks that should be solved. Is it that "the sky is the limit" in development of the livestock production and production of food in general in order to provide food for growing global population, or that the resources that can be used in development of agriculture and other intensive productions are already seriously limited and devastated? Finding the true balance between those directions equally important for future progress is one of the biggest challenges in front of the scientific community and leading in new innovations and generating development trends. There were already many answers in scientific world, but also many new questions. A range of research starts from the appropriate models of livestock production to reduce poverty and hunger of the most vulnerable populations in Africa (*FAO, 2012*), to the development of models using insects as human food and animal feed, so-called mini-livestock (*FAO, 2013*), or the world's first "test-tube burger", still very strange and expensive *in vitro* model of meat production from the Dutch laboratory (*Duhaime-Ross, 2013*). Between these basic and pioneering efforts still prevail and dominate the researches in order to improve conventional, intensive livestock production and products.

Current innovations and possible trends in development of intensive livestock production

First, of course, are the changes in animals themselves. The increase of genetic potential and production performance of farm animals is obvious, but not only in sense of increase of production of meat, milk or eggs, but also in sense of increased economic efficiency, reproduction, animal health, application of biotechnological procedures, etc. Changes are most obvious in the most intensive livestock fields, such as poultry production, pig production or dairy production. For example, production performance of modern hybrid laying hen produced and spread worldwide by only few multinational breeding companies is already very close to biological maximum (one egg per hen every day), in pig production use of

hybrid pigs is increasingly being promoted, or in dairy production one bull can have tens of thousands of progeny worldwide through artificial insemination. On the other hand, livestock production continues to threaten natural biodiversity and genetic resources (*Lukić et al., 2006; Hoffmann, 2010; Tixier-Boichard et al., 2012)*. Promising techniques in further development of genetic potentials of farm animals are currently based on genomics (and other "omics") and biotechnology progress.

Animal nutrition is progressing almost with the same intensity as the genetic improvement, and regarding its crucial importance in livestock production, it is the only way to maintain productivity, product quality and health of animals. Traditionally, there are numerous innovations in animal nutrition, starting with use of new products and additives, through innovations in processing, analysis of food, to equipment and feeding (*Lukić et al., 2009, 2011; Lukić et al. 2012)*. Preoccupation of animal nutritionist shifts from exact evaluation of nutritional quality of various feedstuffs and needs of animals (which are already well known) to a concern about the effect of feed on health, performance of animals and animal products, and to nutrigenomics. But paramount challenge for researchers will be how to increase crop production and change common feeding practise to solve food/feed/fuel competition, especially in case of predicted growth of poultry and pig industry and their needs for corn and soybean.

The innovations in livestock production technology and equipment are usual, and are following the general technological development. More and more we see robots in production, primarily in most labour intensive jobs such as milking cows, cleaning boxes with slate floors or feeding. Very obvious is penetration of information technologies into livestock production, equally to farms as well as to fields, primarily in form of sensors, software applications and models to record/analyze/control every aspect of production, including physiological parameters of animals (*Gebbers and Adamchuk, 2010*). Simply, this trend follows famous management (and research) quote: "If you cannot measure it, you cannot manage it". Also, great changes in equipment and production systems are induced by new regulations related to animal welfare, food security and ecology, especially in Europe.

More and more, special focus is on links between livestock, ecology and bio-energy. Livestock industry is often criticized for its contribution to air and water pollution. Also, strict new ecology regulations and higher bio-energy demand as a response to climate changes, is current state in many developed countries. On big farms biotechnology applications and integrated manure management system are gradually replacing conventional waste management practise. Anaerobic digestion system especially has become an attractive method of treating animal wastes due to its capabilities of stabilizing the wastes, reducing odours and

recovering energy, on farm scale or as centralized biogas plant (*Umetsu et al., 2005, Umetsu et al.2011).*

In general, we can see in researchers efforts and production sector changes two new parallel trends and directions of development: ecological and energy sustainability (i.e. "sustainable agriculture") and qualitative and quantitative efficiency (i.e. "precise agriculture"). Both directions are aimed at increase of production with preservation of existing resources. In countries with most intensive livestock production this can be seen from the trend of decrease of total number of some farm animals, with simultaneous increase in total production of animal products.

It is clear that huge incentive to future development of livestock production lay in investing into research, commercialization of results and fast and effective transfer of new solutions to producers.

The present and future livestock production in Serbia

Serbia has close to 9 million hectares of surface (88 509 km^2), 5.1 million ha are agricultural land, of which about 1.4 million ha are pastures and meadows. First preliminary statistical results from the Census of Agriculture 2012, shows that Serbia has currently the total utilized agricultural area of 3.36 million ha of its territory (*Statistical Office of the Republic of Serbia, 2013)*. Natural resources, above all respectable quantity and quality of agricultural land and favourable climate allow development of all types of agricultural productions in Serbia.

Almost half of the 7.2 million residents of Serbia are located in rural regions, and approximately one quarter of the population in Serbia is working in the field of agriculture. There are 631,122 agricultural holdings in Serbia and almost all (99.6%) are family agricultural holdings. On average, Serbian agricultural producer is a family farm that utilizes 4.5 ha of agricultural area, owns one tractor and raises 1 head of cattle, 4 heads of pigs, 3 heads of sheep, 26 heads of poultry and 1 colony of bees.

Different conditions and different development of agricultural production are present in different parts of Serbia. Figure 1 shows one possible type of classification, with map of division of Serbia into three different rural regions and urban area (*Bogdanov, 2007*). Each rural region is emphasized by general characteristics in terms of agricultural and livestock production.

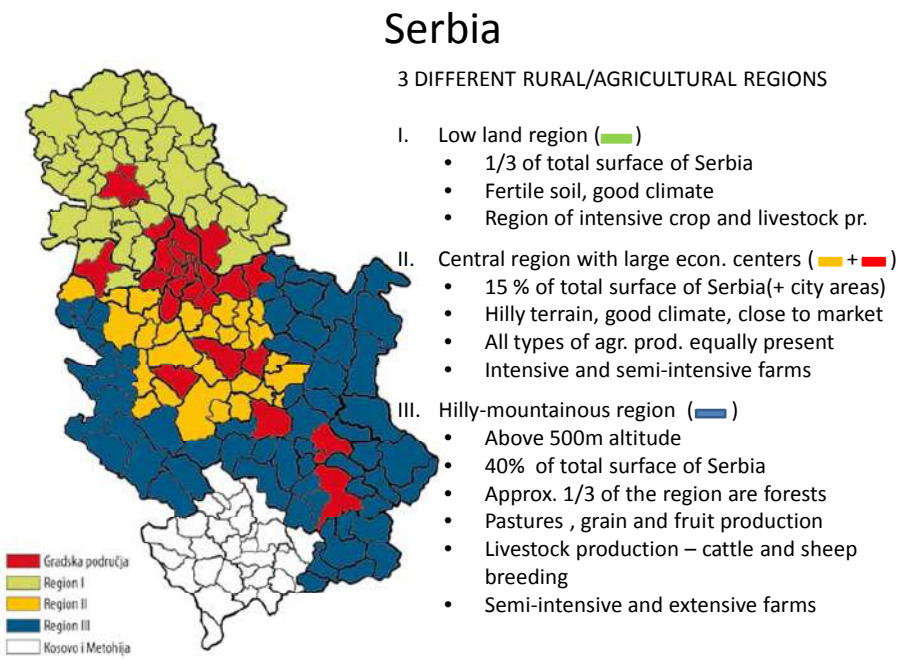


Figure 1. Serbian rural/ agricultural regions and their main characteristics

Field crop and livestock productions are the most common agricultural productions in Serbia, with share of about 40% each in total agricultural production, followed by fruit and vegetable production with share of approx. 12%. Grains, primarily corn and wheat, are most often grown, almost on half of all available fields in Serbia every year. Highly present in the sowing structure in Serbia are also industrial plants, primarily sunflower, soybean and sugar beet. Especially important and well developed is seed production of high quality domestic hybrids and varieties of most crops grown in Serbia.

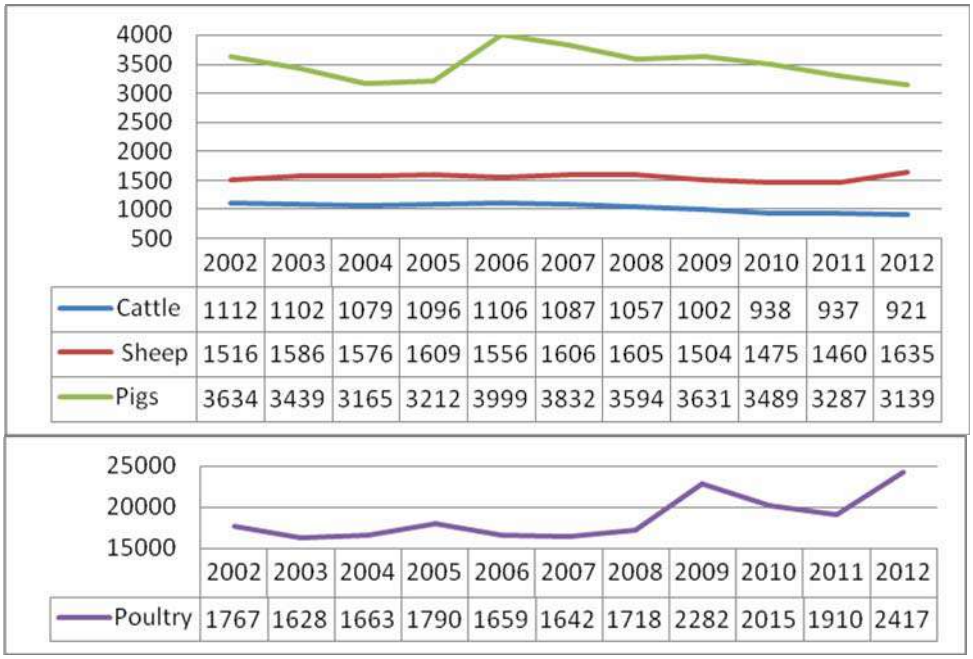
In the recent years, Serbia has become one of the 10 biggest exporters of corn in the world. Also, among other major exported products, are wheat, sugar from sugar beet, sunflower and soybean oil, fruits, seeds and seed material, but also beer and bottled water. Unfortunately, there is no animal product in 10 most important exported agricultural products from Serbia.

Based on above mentioned general facts, it is obvious why in Serbia agriculture has traditionally been one of the major branches of the economy, and considering the natural resources and their potential, agriculture is actually one of the most important branches of economy in the strategy of development of Serbia in future. But, it is also obvious that livestock production in Serbia is far from the

potential and possibilities, concerning all available country recourses for animal production, primarily feed availability. Livestock production in Serbia has been decreasing for over two decades. For example, twenty years ago, when Serbia had double cattle population compared to present situation, one of the major exported agricultural products in country was high quality beef. However, today, situation is much different.

Actual share of livestock production (in year 2012) in total agricultural production is 37.9%. This could be general indicator of relatively poor agricultural development in Serbia, if the criterion is considered that share of livestock production in general agriculture of under 35% indicates an underdeveloped agriculture as a sector in a country's economy. In other words, the natural resources are used in a way that would not bring optimal benefits, or under used, or not used at all, like available grassland in Serbia. Additional information which also can confirm negative trend in agriculture development in general and livestock production particularly in Serbia, is number of livestock unit per ha of agricultural land in years 2007 and 2012, which was 0.31 and 0.28 respectively.

Cattle and pig production are dominant and together make almost 80% of total livestock production in Serbia (43 and 33% respectively in year 2012), and poultry and sheep production have moderate, but stable level of development (16 and 7% respectively in year 2012). Beekeeping, goat farming, fishing and horse breeding are also present in livestock production in Serbia, but to a lesser extent. The graph 2 shows trends in total number of heads of major types of farm animals in Serbia over the last 10 years. It can be observed that the number of heads of farm animals in Serbia has been decreasing in general, especially cattle by 2-3% per year in the last decade, accompanied by reduced production of animal products.



Source: Statistical Office of the Republic of Serbia

Graph 2. Total number of heads of major farm animals in Serbia in period 2002-2012 (thousand heads)

Serbia is currently on the edge of self-sufficiency in production of meat, and covers the needs of its population from own production, but have small export surpluses. However, the projection of the trend in production and consumption of beef meat in Serbia shows that in next few years it will become import dependant if the trend of decrease of heads of cattle continues in future (*Ivkov et al., 2012*). Like in case of analysis of production of beef, it can be observed that production and consumption of pork in Serbia are almost equal and that very soon country will become import dependant, which is especially unacceptable because basis for fast and high development of this production is very strong. Similar situation is in poultry sector in Serbia (*Milošević et al., 2011*).

A relevant element for fast and efficient development of livestock production is increase in number of large livestock farms, especially those that correspond in size to industrial facilities in Serbia. On these farms is the only cost-effective and efficient implementation of some new technologies, such as for example the biogas plants. According to our estimates, in Serbia the number of such industrial livestock producers is very low, just over 10% of pig and poultry

production, while in cattle production, it is estimated that there are less than 5% of the total number of larger livestock farms (*Lukic, 2012*). Precise data on this and many other issues are expected to be available upon publication of the results of Agricultural Census performed in Serbia in year 2012.

Agricultural legislation in Serbia is already very similar to that in EU, meaning very strict and producer demanding especially in animal production sector, with obligation that during the current process of integration of Serbia in EU, all EU legislation will be adopted and implemented. Legal obligations of animal producers are already present to ensure health safety of animals and products, environment and animal welfare protection in whole production process, but yet without serious help and financial governmental support. Also is very important to point out that, considering that in EU legislation and policy related to agriculture are currently introduces important changes that will be implemented in the period 2014-2020, transposition of those new requirements have yet to be started in Serbia after the adoption process be finished in EU (*Jukna, 2013)*.

If we analyze livestock production in Serbia taking into consideration all aspects, results of this analysis indicate several key characteristics of present situation in livestock production in Serbia, as well as consequences of this situation, problems and issues that need to be solved (table 1).

Table 1. Current state/problems in livestock production in Serbia

Key characteristics of present situation	**Consequences of present situation**
• Constant drop in number of animals	➢ **Unused resources**
• Stagnation in farm production efficiency, increase of prices of animal feed and energy, lower profitability in this production	➢ **Constant drop in number and size of farms and farmers engaged in livestock production**
• Consumers' concern and legal obligation of the producer to ensure health safety of animals and products, environment and animal welfare protection in whole production process	➢ **More complex and difficult general conditions for livestock production and food production**
• Necessity of development of modified and new ways of livestock production systems according to changed "rules of the game"	➢ **Lack of national knowledge, research and development in this direction**

Knowing current state and problems in livestock production in Serbia and future way of development of livestock production worldwide, in general core tasks can be defined, some potential directions for solving problems, and who will be in charge for this in Serbia (table 2). Of course, prerequisite for this is that state, i.e. government, should take the leading role in the future development of livestock production with its regulatory measures. A good step in this direction is the design of the umbrella document, the Strategy for development of agriculture and rural areas of the Republic of Serbia for the period 2014-2024, which is in progress (*Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia, 2013*). However, it still remains as the most important task, to take a course of development in line with global and regional trends in livestock production and adapt to our specific environment, capacities and specificities, to include all structures and ensure continuity in the implementation of the defined process development in livestock production.

Table 2. Potential directions for solving problems in livestock production and job holders

• **Government/State**
– To preserve number of animals and attract agricultural producers to engage more in this difficult job, but in a modern way
• **Science**
– To increase the quality of livestock (genetics and reproduction) – To improve technologies, primarily in regard to • Health protection • Animal nutrition • Energy efficiency (bio-fuels, bio-mass, bio-gas) • Ecology
• **Producers, science, extension service and government**
– To harmonize production systems with new regulations and demands, to introduce standards in the fields of food safety, ecology and animal welfare, to spread domestic (custom-made) technologies on livestock production and products

Finally, it seems that only right direction is **sustainable intensification of livestock production** in Serbia, and role of science and research in this should be very important, like everywhere in progressive parts of the world. Livestock production in Serbia has significant potential for development in the future, but it must be expected that this process would be long and complex, especially considering number and farm size of livestock producers in country.

Conclusion

The fact is that livestock can feed people, land and be a source of energy in a sustainable manner. This was usually common case in some old, traditional, non-industrial ways of productions, so it can be promising solution for future on modern way. Development of those modern, effective and sustainable solutions should be our task. However, given that livestock, like any other production are developed by humans, global and local human community decisions remain crucial to their current and future fate.

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Stočarstvo danas i sutra – studija slučaja Srbije

M. Lukić, Z. Pavlovski, Z. Škrbić, K. Umetsu, V. Petričević

Rezime

Studija sumira sadašnje i buduće izazove stočarske proizvodnje u svetu i ukazuje na postojeće inovacije i trendove u stočarskoj proizvodnju u celini. Posebno se analizira stanje i perspektiva razvoja stočarske proizvodnje u Srbiji. Rad ukratko prikazuje trenutno stanje u poljoprivredi i stočarskoj proizvodnji u Srbiji, identifikuje osnovne karakteristike i glavne probleme trenutnog stanja, kao i potencijalne mogućnosti i nužne korake za rešavanje evidentnih problema u kojima je stočarska proizvodnja Srbije danas, pre svega uzimajući u obzir buduće pravce razvoja stočarstva u svetu.

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GENETIC DIVERSITY OF BREED OF BEEF CATTLE POPULATION CONCERNING MEAT PRODUCTION AND QUALITY

V. Jukna, Č. Jukna, E. Meškinytė – Kaušilienė

Laboratory of Meat Characteristic and Quality Assessment, Lithuanian university of health sciences, Veterinary Academy; Tilžės 18, LT-47181 Kaunas; Lithuania. Phone: (8~37) 36 34 14; el.
Corresponding author: vjukna@lva.lt;
Invited paper

Abstract: The research presents comparative meat production and quality research data concerning Lithuanian black and white (LBW) and Lithuanian red (LR) as well as the breeds, used for improving the Lithuanian ones such as Holstein (H), Swedish White (SW) and the most frequent beef cattle populations in Lithuania Charolais (CH), Limousines (LI), Aubrac (AU), Simmental (SI) and Angus (AN). The start point for testing the dairy bulls was 120 days and for beef bulls was 200 days. All the bulls were grown at the controlled feeding station under the same conditions until the age of 500 days. At the age of 500 days there were selected 12 bulls representing each breed, controlled slaughters were made and samples were taken from the musculus longissimus dorsi at the two last ribs for meat quality research. It was stated that the weight of all dairy bulls was alike at the end of testing period. LBW bulls consumed 6.1 ME MJ (metabolizable energy MJ) or 8.2 percent less for each kilo gain than LR breed bulls. The breed impact was observed on the carcass yield as well. H bulls carcass yield was smaller than LWB by 1.75 percent ($p<0.05$). The other breed carcass yield was similar to the LWB bulls. The least musculus longissimus dorsi cross-sectional area was observed in H bulls ($p<0.05$). The breed influenced the meat chemical composition as well. SZ bulls meat contained most protein and intramuscular fat ($p<0.05$). H meat had lower water binding capacity and higher cooking loss ($p<0,01$ and $<0,001$). Indexes of meat quality differences concerning dairy breeds were statistically unreliable ($p> 0.05$). Concerning beef bulls breeds the highest weight was observed in CH and SI bulls at the age of 500 days. It was higher than AN bulls by 60.6 and 59.4 kg or 10.0 and 9.8 percent ($p<0.05$ ir <0.01). The feed costs of weight gain differences between breeds have not been identified. The highest carcass yield was observed in CH and the smallest in AN and SI breed bulls. The difference amounted to 4.7 and 3.4 percent ($p<0.05$). French breed carcass yields were ranked one conformation class higher than the other breeds. CH musculus longissimus dorsi cross-sectional area was larger than AN bulls by 25 cm2. Chemical composition of all breed's bulls meat was similar, excluding the amount

of intramuscular fat. Angus and Simmental meat contained the highest amounts ($p<0.05$). Breed influenced meat color as well. Limousine meat was the darkest and Angus bulls meat was the least red ($p<0.05$). Limousines meat had the highest water binding capacity and the lowest cooking loss, Simmental had the lowest water binding capacity ($p <0.05$) while Angus had the highest cooking loss ($p<0.01$). Angus meat was the softest and Aubrac and Simmental meat were noticed to be the hardest ($p<0.05$). Concerning meat protein sufficiency ratio indices all breed's meat is noticed to be similar, excluding Aubrac breed and bulls' meat meets the requirements of high biological value meat.

Key words: breed, bulls, meat quality, diary bulls, beef bulls.

Introduction

Bovine meat is an important source of protein, minerals, trace elements and vitamins in human nutrition. Its nutritional value depends on easily assimilated protein, well-balanced ratio of amino acids, unsaturated fatty acids ratio, connective tissue structure, minerals, trace elements, vitamins and other substances quantities and ratio (Gutzwillera et al., 2003; Purslow, 2005). Biological value of bovine meat is determined by essential amino acid and lipid structure (Serra et al., 2004; Raes et al., 2004). Bovine meat quality is described by the chemical composition and physical properties (Fischer, 2002; Kessler et al., 2006). Physical meat properties are determined by evaluating color, pH, water binding ability, hardness and cooking loss during processing (Boles et al., 2005; Viera et al., 2007). Bovine meat in Lithuania derives mainly from dairy breed cattle offspring and culled defective adult cattle. In Lithuania beef livestock development begins after 1995, with more frequent crossbreeding of dairy breed cows with beef breed bulls was applied. However, purebred and crossbred beef breed cattle represent only 8 percent in Lithuania as traditionally diary breed selection programs were pursued with respect to beef meat quality properties (Юкна; 1974; Banys, 1988; Jukna, 2006). The main dairy cattle breed meat production in Lithuanian and its quality at various time periods was practically the same, although different breed were used to improve it during the selection process (Станкявичюс, 1975; Zostautiene et al., 1994; Jukna et al., 2006). Recently, country has increased Holstein and Swedish White populations. Therefore, in order to foresee the selection directions of Lithuanian Black and White and Lithuanian Red breeds in the future it is imperative to have these breed's comparative data concerning assessment of meat production and quality. Lithuania has developed purebred beef cattle populations although it is not numerous. More numerous breeds are as follows Charolais,

Limousine, Aubrac, Simmental and Angus. The comparative data concerning assessment of meat production and quality is necessary when deciding on the development, accordingly to the individual features of country's regions.

Materials and methods

Research objective is comparative assessment of meat production and quality concerning Lithuanian modern selection main dairy cattle breeds and the most numerous beef cattle breeds. Groups with non-castrated bulls were formed representing Lithuanian Black and White (LBW), Lithuanian Red (LR) and the breeds, used for improving the Lithuanian breeds as Holstein (H), Swedish White (SW) and the most frequent beef cattle population Charolais (CH), Limousin (LI), Aubrac (AU), Simmental (SI) and Angus (AN) in order to do the research. All the bulls in the groups were grown at the same time in the controlled feeding station (UAB <Šilutės veislininkystė>) /JSC <Silutes stockbreeding>/ on identical feeding and housing conditions.

Testing dairy breed bulls started at the age of 120 days and testing beef breed bulls started at the age of 200 days. All the bulls were grown until the group reached an average age of 500 days. Groups contained from 13 to 35 bulls. Each breed variety was represented by offspring of two - four bulls. Bulls were kept tethered. Concentrated forage was rationed and grass feeding was not limited for all groups' bulls. Quantities of ingested feed were determined by controlled weighing the given feed and the residues of uneaten feed every two weeks for two consecutive days and that way the estimated amount of ingested feed was calculated. Bulls were weighed individually prior to starting the experiment at the age of 120 or 200 days and at the age of 500 days. When the groups reached an average age of 500 days 12 bulls from each group were selected as most likely representing the group average and controlled slaughters were performed. The carcass weight, conformation and fatness class (according to EUROP standards), carcass hip-thigh weight, morphological composition of the hip-thigh and the cross-sectional area of the longest back muscle at the two last ribs (Musculus longissimus dorsi) were determined during the slaughter. In order to assess the meat quality a sample was taken from the longest back muscle at the two last ribs (Musculus longissimus dorsi). The meat was matured for 48 hours within a temperature of 4 oC. The chemical composition and the physical properties of meat were evaluated as follows: dry substance (using automatic scales "SM-1"), quantity of ash, by burning organic matter until a constant weight at the temperature of 600 - 800 oC, pH (using pH-meter INOLAB 3), coloring (using Minolta 410 Chrometer the measurements were done on color luminosity L *, redness a *, yellowness b *), wateriness (by measuring the sample weight loss within 24 hours.), water binding

capacity by applying Grau and Hamm method (1953), subsequently enhanced by Hamm (1972) compaction method; hardness by applying Warner- Bratzler method (Bratzler, 1949), the cooking loss by applying Shillings method (Schiling, 1966), fat content by applying Soxhlet method (Soxhlet, 1879), protein quantities by applying Kjeldahl method (King-Brink, Schranek, 1993), tryptophan quantities by applying Spies and Chambres method (Spies, 1967) and oxyprolin quantities by applying Neuman and Logan method (Neuman, Logan, 1950); meat protein sufficiency ratio was determined by the amino acids tryptophan and oxyprolin ratio. Data was processing by applying statistical package R, version 2.0.1. (Gentlemen, Ibaka, 1997). Reliability on differences was determined by applying Stjudent. Differences considered being statistically reliable when p-0, 05.

Results and discussions

Experiments done on dairy breeds illustrated that at the age of 500 days the SW bulls weight was 16 kg or 3.35 percent more than LBW bulls (p> 0.05) (Table 1).

Table 1. Lithuania the most common breeds of dairy bulls meat production

Indexes	Lithuanian black and white (LBW)	Lithuanian red (LR)	Holstein (H)	Swedish White (SW)
Weight 500 d. age, kg	476.5	473.3	475.5	492.5
Weight gain during the period from 120 – 500 d. age, g	989	987	970	1029
Feed cost kg weight gain, MJ AE	74.2	80.3	74.1	77.4
Pre-slaughter weight, kg	482.3	483.7	485.0	497.0
Carcass weight, kg	251.1	247.6	243.1	254.7
Carcasse				
Yield, %	51.87*	51.19	50.12*	51.25
Conformation class	O	O	O	O
Fat class	2.4	2.5	2.8	2.9
Ham yield, %	33.0	32.2	31.3	31.85
Ham soft parts yield, %	79.3	80.4	80.0	78.9
Long muscle cross-sectional area, cm2	78.9*	72.1	71.6*	78.0

*p<0.05

The weight of remaining breeds bulls was similar to LBW bulls. LR bulls consumed 6.1 ME MJ (metabolizable energy MJ) or 8.2 percent more for each kilo gain than LBW breed bulls. The differences of feed consumption of weight gain among other breeds were not important. The least carcass yield was in H bulls. It was smaller than in LBW bulls by 1.75 percent (p <0.05). Other bulls' carcass yields were similar to that of LBW. The carcass conformation class was the same for all the bulls. LBW had the lowest fatness class and the SW had the highest

fatness class (p> 0.05). Carcass hip thigh yield was the largest in LBW and the lowest in H bulls. The LBW had the largest musculus longissimus dorsi cross-sectional area and H bulls had the smallest cross-sectional area. The difference amounted to 7.3 cm2 (p <0.05) (Table 2).

Table 2. Lithuania the most common breeds of dairy bulls meat quality (musculus longissimus dorsi)

Indexes	Lithuanian black and white (LBW)	Lithuanian red (LR)	Holstein (H)	Swedish White (SW)
Dry matter, %	24.16	23.47	24.86	26.69*
Fat, %	1.73**	1.57	2.27*	2.80*
Protein, %	21.33	20.78	21.55	22.85
Ash, %	1.10	1.12	1.04	1.04
pH	6.21	6.32	5.85	6.68
Colour:				
L*	35.47	35.12	37.15	36.12
a*	17.42	17.52	16.40	17.83
b*	4.44	4.27	5.02	4.67
WHC, %	61.50**	60.97	53.9**	54.06**
Cooking loss, %	18.90***	19.28	39.75***	33.30***
Shear force, kg/cm2	1.99	1.78	2.16	2.19
Tryptophan, mg/%	316	357	285	305
Oxyprolin, mg/%	62.9	68.7	53.5	57.7
Meat protein value,%	5.02	5.20	5.32	5.28

Some meat quality data differences were observed depending on breed impact. SW bulls meat contained more dry substance than LBW bulls meat by 2.53 percent (p <0.05). SW bulls meat contained most intramuscular fat as well. The difference amounted to 1.07 percent compared with LBW (p <0.05). Meat pH, color L *, a *, b * differences between breed were not statistically significant (p> 0.05). SW and H meat had lower water binding capacity and significant higher cooking loss (p <0.01 and <0.001) compared with LBW. Meat toughness was similar in all breeds. Concerning meat protein sufficiency ratio all breed's meat meets the requirements of high biological value meat. The differences regarding this index between breed were marginal.

The breed's impact to beef breed bulls' weight was significant (Table 3).

Table 3. Lithuania the most common varieties of beef cattle meat production

Indexes	Charolais (CH)	Limousines (LI)	Aubrac (AU)	Simmental (SI)	Angus (AN)
Weight 500 d. age, kg	608.7**	570.0	563.7	607.5*	548.1**
Weight gain during the period from 120 – 500 d. age, g	1166	1076	1034	1071	1024
Feed cost kg weight gain, MJ AE	73.2	68.7	71.4	70.6	70.15
Pre-slaughter weight, kg	617.0	575.0	571.7	609.8	552.0
Carcass weight, kg	358.9	323.7	329.3	334.2	295.3
Carcasse					
Yield, %	58.2*	56.3	56.0	54.8*	53.5*
Conformation class	U	U	U	R	R
Fat class	2.7	2	2	2	2.1
Ham yield, %	34.4	33.8	32.7	33.5	32.9
Ham soft parts yield, %	82.1	83.5	82.7	80.5	81.8
Long muscle cross-sectional area, cm2	109**	89.0	84.0**	92.0	87.0*

*p<0.05; **p<0.01

The highest breed impact was observed in CH and SI, and the lowest one in AN bulls. The difference amounted accordingly to 60.6 kg and 59.4 kg or 10.0 and 9.8 percent. ($p < 0.05$ and < 0.01). Other breeds bulls took an intermediate position regarding the weight. The least feed consumption for the weight gain was observed in LI and the most in CH breed bulls feeding. The difference amounted to 4.5 ME MJ or 6.5 percent. Other breeds bulls feed consumption ratio was similar. The breed's impact to carcass yield was significant. The highest carcass yield was observed in CH and the least in AN and SI breed bulls. The difference amounted to 4.7 and 3.4 percent ($p<0.05$). Other breeds bulls took an intermediate position regarding the carcass yield. French breed carcass yields were ranked conformation class U, while Si and AN breeds were ranked one class lower. CH breed bull carcass fatness was observed to be the closest to the base class (3). Other breed's bull carcass fatness was similar and differed too much from the base class. The largest hip thigh part of the carcass yield was determined in CH and the least in AU and AN breed bulls. The difference was 1.7 and 1.5 percent ($p > 0.05$). The largest soft part of the yield was observed to be in LI, and the lowest in SI breed livestock. The difference amounted to 2.2 percent ($p > 0.05$). The differences regarding this index between other breed were insignificant. The cross-sectional area of musculus longissimus dorsi was significantly larger in CH than other breed bulls. The least index was observed in AU breed bulls. The difference amounted to 25 cm2 ($p<0.01$). Other breeds bulls data concerning this index had fewer differences (Table 4).

Table 4. Lithuania the most common beef breeds of cattle meat quality

Indexes	Charolais (CH)	Limousines (LI)	Aubrac (AU)	Simmental (SI)	Angus (AN)
Dry matter, %	25.05	25.10	25.50	26.00	25.21
Fat, %	1.38*	1.57	1.40	2.20*	2.35*
Protein, %	22.55	23.21	23.02	22.75	22.31
Ash, %	1.12	1.10	1.10	1.10	1.13
pH	5.58	5.91	5.81	5.45	5.48
Colour:					
L*	39.83*	35.63*	39.13	39.75	39.35
a*	23.49	18.41**	29.63**	18.21*	19.75
b*	8.95*	5.92*	7.20	7.42	7.62
WHC, %	54.55	58.53*	52.0	49.7*	53.05
Cooking loss, %	23.90	15.62**	24.4*	21.7	26.80**
Shear force, kg/cm2	1.95	2.00	2.63*	2.50*	1.68*
Tryptophan, mg/%	310.0	318.0	292.0	309.0	318.0
Oxyprolin, mg/%	56.60	54.63	59.42	56.23	55.32
Meat protein value,%	5.20	5.82	4.91	5.51	5.70

*p<0.05; **p<0.01

The differences concerning meat chemical composition among the breeds, except for intramuscular fat quantities, were not statistically significant. AN and SI breeds meat contained the most intramuscular fat and CH bulls meat had the least amounts. The difference amounted to 0.97 percent ($p<0.05$). The breed influenced the bulls' meat color as well. The darkest color of meat was observed in LI and the lightest red color in AU bulls ($p <0.05$). Limousine meat was marked as having lighter yellowness ($p <0.05$). The highest water binding capacity was noted in LI, and the lowest in SI breed meat. The difference was 4.85 mg% ($p <0.05$). The differences according this index among other breeds were less pronounced. The biggest meat cooking loss was observed in AN while the lowest in LI breed bulls whose meat is characterized having the highest water binding capacity. The difference was 7.62 mg% ($p <0.01$). An important meat quality indicator is its hardness. The softest meat was noted AN and the hardest AU and SI bulls meat. The difference was 0.95 kg/cm2 and 0.82 kg/cm2 ($p <0.05$). Meat protein sufficiency ratio meets the requirements of high biological value meat concerning all breed's bulls, except for Aubrac.

The meat production and quality data differences depending on breed impact were discovered by other scientists as well. Golz et al. (2002) compared the quantities of protein in meat of Simmental, Limousin, German Angus and Galloway cows and noticed that Simmental had the biggest amount and German Angus breed had the least amount of protein quantities. During other experiments Golz et al. (2009) compared the Simmental, Limousine and Angus bulls meat's

physical characteristics and noticed cooking, baking, color L * and hardness differences between breeds. Martin (2010) compared meat quality of Charolais, Ukermarker, Simmental, Limousin, Hereford, Angus, Aubrac and Salers bulls and discovered that breed had impact on many meat quality indicators. The fact of breed impact on meat quality is analysed and proved by a number of other authors (Скоркина и др., 2004; Dufrasne et al., 2006; Uschakov et al. Levachin et al., 2008).

Conclusions

The weight of all tested dairy bulls was alike at the age of 500 days. Lithuanian Black and White bulls consumed 6.1 ME MJ or 8.2 percent less for each kilo gain comparing to Lithuanian Red breed bulls. The feed costs of weight gain of other breeds were similar to Lithuanian Black and White.

The breed had an impact on the carcass yield, but did not have impact on carcass conformity and fatness classes. Holstein carcass yield was smaller than Lithuanian Black and White by 1.75 percent ($p<0.05$). The other breed carcass yield was similar to the Lithuanian Black and White. The largest cross sectional area of musculus longisismus dorsi was in Lithuanian Black and White, while the smallest in Holstein ($p<0.05$).

The breed had an impact on some meat quality indexes. Regarding dairy breeds Swedish White bulls meat contained the most dry substances and the most intramuscular fat ($p<0,05$). Holstein meat was noticed having lower water binding ability and higher cooking loss ($p<0,01$ and $<0,001$). Indexes of meat quality differences concerning other breeds were statistically insignificant. Concerning beef bulls breeds at the age of 500 days the highest weight was observed in Charolais and Simmental bulls. CH and SI weight was higher than Angus bulls by 60.6 and 59.4 kg or 10.0 and 9.8 percent ($p<0.05$ and <0.01). The feed costs of weight gain differences between breeds have not been identified. The breed's impact on the carcass yield is of important significance. The biggest carcass yield was observed in CH and the smallest in Angus and Simmental breed bulls. The difference amounted to 4.7 and 3.4 percent ($p<0.05$). French breed carcass yields were ranked conformation class U while the other breeds class O. The impact of breed was noticed on the hip thigh and its soft parts yield as well. The difference between breeds concerning musculus longissimus dorsi cross-sectional area amounted to 25 cm2 ($p<0.01$)..Charolais had the largest and Aubrac breed bulls had the smallest cross-sectional area.

Chemical composition of all breed's bulls meat was similar, excluding the amount of intramuscular fat. Angus and Simmental meat contained the highest amounts ($p<0.05$). Breed influenced meat color as well. Limousine meat was the

darkest and Angus bulls meat was the least red ($p<0.05$). Limousines meat had the highest water binding capacity and the lowest cooking loss, Simmental had the lowest water binding capacity ($p <0.05$) while Angus had the highest cooking loss ($p<0.01$). Angus meat was the softest and Aubrac and Simmental meat were noticed to be the hardest ($p<0.05$). Concerning meat protein sufficiency ratio indices all breed's meat is noticed to be alike, excluding Aubrac and all breed's meat meets the requirements of high biological value meat.

Genetička raznovrsnost rasa tovne populacije goveda sa stanovišta proizvodnje i kvaliteta mesa

V Jukna, Č. Jukna, E. Meškinytė – Kaušilienė.

Rezime

U radu su izneti rezultati komparativne analize proizvodnje i kvaliteta mesa u okviru istraživanja o litvanskoj crno - beloj (LBW) i litvanskoj crvenoj (LR) rasi, kao i rasama, koje se koriste za unapređenje poput holštajna (H), švedske bele (SW) i najčešćim rasama junadi u litvanskoj populaciji: šarole (CH), limuzin (LI), aubrac (AU), simentalske (SI) i angus (AN) rase. Početni uzrast za ispitivanje mlečnih bikova bio je 120 dana, a tovnih bikova 200 dana. Svi bikovi su odgajani u testnim stanicama pod istim uslovima do uzrasta od 500 dana. U uzrastu od 500 dana izabrano je 12 bikova kao predstavnika rase, koji su zaklani pod kontrolisanim uslovima nakon čega su uzeti uzorci *musculus longissimus dorsi* sa poslednja dva rebra za istraživanje kvaliteta mesa. Konstatovano je da je težina svih mlečnih goveda bila slična na kraju probnog perioda. LBW bikovi konzumirali su 6,1 ME MJ (metabolička energija MJ) ili 8,2 odsto manje za svaki kilogram prirasta od LR rase bikova. Rasa je imala primetan uticaj na prinos trupova. Randman trupova H bikova je bio manji od LBW za 1,75 % ($p <0,05$). Prinos trupa drugih rasa je bio sličan LBW bikovima. Najmanji presek *musculus longissimus dorsi* zabeležen je kod bikova H rase ($p <0,05$). Rasa je takođe uticala na hemijski sastav mesa. Meso LR bikova sadržalo je najviše belančevina i masnog tkiva ($p <0,05$). Meso H rase je imalo manju sposobnost vezivanja vode i veći kalo kuvanja ($p <0,01$ i $<0,001$). Indeksi kvaliteta mesa odnosno razlike između nih, kod mlečnih rasa nisu bile statistički značajne ($p > 0,05$). Kada su tovne rase u pitanju, najveća masa je registrovana kod CH i SI bikova starosti od 500 dana. Telesna masa bila je veća nego kod AN bikova za 60,6 i 59,4 kg ili 10,0 i

9,8 % ($p < 0,05$ i $< 0,01$). Troškovi ishrane u prirastu težine odnosno razlike u ovom parametru, nisu utvrđene. Najveći randman trupa zabeležen je kod CH bikova, a najmanji kod bikova SI rase. Razlika iznosi 4,7 i 3,4 % ($p < 0,05$). Randmani trupa kod francuskih rasa su rangirani za jednu klasu više od drugih rasa. Presek *musculus longissimus dorsi* kod CH bio je veći nego kod AN bikova za 25 cm^2. Hemijski sastav mesa bikova svih rasa bio je sličan, izuzev u količini intramuskularne masti. Meso angus i simentalske rase sadržalo je najveće količine ($p < 0,05$) intramuskularne masti. Rasa je takođe uticala i na boju mesa. Meso limuzin rase je bilo najtamnije a meso angus rase najmanje crveno ($p < 0,05$).

Limuzin meso imalo je najveći kapacitet vezivanja vode, i najniži kalo kuvanja, a simentalska rasa je imala najmanji kapacitet vezivanja vode ($p < 0,05$), dok je angus rasa imala najveći kalo kuvanja ($p < 0,01$). Angus meso je bilo najmekše, a aubrak i simentalsko meso najtvrđe ($p < 0,05$). Sadržaj proteina bio je sličan u mesu svih ispitivanih izuzev Aubrac rase dok je meso bikova generalno visoke biološke vrednosti.

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THE PHENOTYPIC VARIABILITY OF PRODUCTION TRAITS IN THE POPULATION OF SIMMENTAL COWS

V. Pantelić*, D. Ružić-Muslić, M.M. Petrović, D. Nikšić, D. Ostojić-Andrić, S. Aleksić, M. Lazarević

Institute for Animal Husbandry, Belgrade-Zemun, 11080 Zemun
* Corresponding author:Vlada Pantelić, e-mail: vladap4@gmail.com
Invited paper

Abstract: The breeding objective for the Simmental breed of cattle is to achieve maximum genetic values for economically important traits, in line with the economic effects of genetic improvement, which this breed has achieved in developed countries. The present study included 302 Simmental cows selected as bull dams on the territory of the Republic of Serbia in the course of one year. The impacts of paragenetic factors and lactation on production traits (milk yield and fertility) were examined. Order of lactation influenced highly significant deviations ($P<0.01$) in milk yield, milk fat content, milk fat yield and 4% FCM from the general average, as well as slight variations in the length of gestation and lactation, calving interval and service period, that were not statistically significant ($P>0.05$). Simmental bull dams included in the present study, in the lactation period of 311.45 days, have realized milk yield of 5754.49 kg with 3.98% and 230.24 kg of milk fat and 5755.47 kg of 4% FCM. Average duration of gestation of tested heads was 286.31 days, the service period 110.37 days, and calving interval 398.44 days. Overall comprehension and understanding of a number of paragenetic factors and their interactions on specific production characteristics can have impact on improvement of milk yield and fertility in cows.

Key words: fertility, milk yield, Simmental breed, lactation.

Introduction

Simmental cows in the Republic of Serbia are reared in semi-intensive conditions of housing, care and feeding, or on small farms with a few animals, to farms with dozens of breeding animals in a more intensive system of rearing. Genetic improvement of Simmental cattle in the country is achieved by selection and breeding, in a pure breed. At one time there were attempts to introduce genes of red

Holstein breed to improve milk production traits and milkability (*Petrović, M.M. et al 2009*).

The necessity of continuous improvement of the production of milk, milk fat and milk proteins imposes the need for systematic work on all levels related to the aforementioned production. Their general characteristic is that they are quantitative genetic traits and their degree of phenotypic expression depends on the interaction of genotype and environment in which production takes place. In order to achieve high milk and milk fat yield it is needed, in addition to a strict selection process, to provide adequate production conditions, particularly with regard to nutrition, then rearing and care (*Pantelić 2006*).

By examining the production potential of first calving Simmental cows calved in the period 2007-2010 on individual farms in Serbia, *Nikšić et al. (2011)* found that their average milk production of 4348 kg of milk with milk fat content of 3.93% and milk fat yield of 171.1 kg. With the exception of year 2009, milk yield in first calving heifers continuously increased on average by about 100 kg of milk per animal per year.

Panić and Vidović (2006) have conducted study of the heritability of important milk traits of of Simmental cows on two farms in Vojvodina. First lactation lasted in average a long time - 350 days, as a consequence of late first insemination on these farms. Cows realized in standard lactation average milk production of 4298 kg, with the milk fat yield and content of 170 kg and 3.98%, respectively. Average yield of 4% FCM was 4277 kg.

Reproduction in intensive cattle production is the most important phase because it provides animals for renewal of the herd, milk production and fattening, i.e. meat production.

Croatian Livestock Selection Center (2003) provides some indicators of the reproduction of Simmental cattle population. The average age of registered cows in first lactation was 28 months, and the duration of the service period 120 days.

Bielfeldt et al. (2004) have established the average duration of service period of 92.9 days and of the calving interval 384.5 days. They have also concluded that the year and season of calving have a highly significant effect ($P<0.01$) on the duration of service period and calving interval.

Given the high economic importance of fertility traits in cattle it is necessary to know certain factors affecting fertility in cows. The fertility of cows is much more influenced by environmental factors because most of the variation of reproductive traits are affected by them. When analyzing individual factors, their impact on the fertility of cows is different, and also complex action is possible. More comprehensive knowledge of the impact of certain factors on specific reproductive traits may improve fertility in cows (*Trifunović et al., 2004).*

Petrović M. D. et al. (2005), have analyzed the effect of paragenetic factors on production traits in complete lactations in Simmental cows. The influence of lactation on all production traits was highly significant, $p<0.01$ with the exception of duration complete lactations where the impact was not statistically significant, $p> 0.05$.

Material and methods

Simmental breed is the breed most present in Serbia, where the total number of cattle population is about 908,990 animals, of which about 525,000 cows and heifers. In the breed structure of cattle in Serbia, it is estimated that the Simmental breed makes up about 80%, the group of Black and White Holstein-Friesian cattle around 10%, while the primitive breeds and cross-breeds account for about 10% of the total number of cattle. Active population of Simmental cattle in the Republic of Serbia comprise only animals that are included in the main animal recording/registration system (about 90,000 head or 17%). The breeding objective for the Simmental breed of cattle is to achieve maximum genetic values for economically important traits in line with the economic effects of genetic improvement, which this breed has achieved in developed countries *(Pantelić et al. 2011).*

This study included 302 Simmental cows selected as bull dams on the territory of the Republic of Serbia in the course of one year. Selection of cows into the herd of bull dams was performed after completion of the first, and based on the subsequent lactations.

The study included the following properties of milk throughout the lactation:

- Duration of lactation, days
- Milk yield, kg
- Milk fat content,%
- Milk fat yield, kg
- Yield of 4% FCM, kg

Correction of milk yield at 4% FCM was performed using the Gaines-Davidson's formula:

$4\% FCM = 0.4 M + 15 F$

Where:

M - milk yield

F - milk fat yield

The following reproductive traits were investigated:

- Duration of pregnancy (days)
- Duration of service period (days)
- Duration of the interval between calving (days)

The impact of paragenetic factors and order of lactation on production traits (milk yield and fertility) was examined. Analysis of the impact of paragenetic factors on milk production traits and fertility was carried out by the method of least squares, using a fixed model:

$Y_{ml} = \mu + L_l + e_{ml}$

Where:

Y_{ml} = Expression of the studied trait of cow *m*, which produced in lactation *l*

μ = general average

L_l = fixed effect of *l* order of lactation

e_{ml}= random error

Results and Discussion

Study of the duration of lactation has practical importance because it is through this trait that the utilization efficiency of dairy cows in milk production can be estimated. Duration of lactation is related to the duration of the service period. Earlier drying due to lower milk production leads to the problems in regard to the body condition and at calving. Poor diet does not shorten the lactation but decreases the yield of milk and adversely affects the reproduction. Prolonged lactation indicates that the cows are not inseminated in optimal time, or are dried on time.

Table 1. General average (μ), deviation from the general average (ĉ), least square mean values (lsm) and their errors (S) and significance of studied effects on duration of lactation, days

Effects	**N**	**ĉ**	**Sc**	**lsm**	**Slsm**
General average					
μ	975	311.45	2.24		
Lactations ^{ns}		f_{tab} **= 11,019**		df_1**=6**	df_2**=949**
I	292	-3.66	4.96	307.79	3.01
II	230	-2.31	3.43	309.14	1.74
III	170	-3.49	2.15	307.96	1.54
IV	116	-0.04	1.57	311.41	2.64
V	81	-0.67	2.36	310.77	4.11
VI	48	1.74	3.80	313.19	5.72
≥VII	38	8.42	5.81	319.86	7.84
ns – P>0.05		*** – P<0.05**		**** – P<0.01**	

Table 2. General average (μ), deviation from the general average (ĉ), least square mean values (lsm) and their errors (S) and significance of studied effects on milk yield in complete lactation, kg

Effects	N	ĉ	Sc	lsm	Slsm
General average					
μ	975	5754.49	109.80		
Lactations **		f_{tab}= 17,808		df_1=6	df_2=949
I	292	-927.92	242.56	4826.57	147.48
II	230	-232.67	167.83	5521.82	84.94
III	170	139.15	105.13	5893.64	75.25
IV	116	274.07	76.77	6028.56	129.10
V	81	321.71	115.33	6076.21	200.90
VI	48	301.18	185.86	6055.68	279.63
≥VII	38	124.48	284.25	5878.97	383.58
ns – P>0.05		*** – P<0.05**		**** – P<0.01**	

Based on the results presented in Table 1 it can be concluded that the average duration of lactation period was 311.45 days. Minimum duration of the lactation period was 307.79 days and maximum of 319.86 days.
Similar results were obtained in the research by *Perišić (1998),* and higher and significantly higher values were obtained by *Petrović (2000), Perišić et al. (2002), Panić and Vidović (2006).*

From Tables 2, 3, 4 and 5, it can be concluded that the lactation influenced the incidence of highly significant deviations (P<0.01) in milk yield, milk fat content, milk fat yield and 4% FCM from the general average.

Table 3. General average (μ), deviation from the general average (ĉ), least square mean values (lsm) and their errors (S) and significance of studied effects on milk fat content in complete lactation, %

Effects	N	ĉ	Sc	lsm	Slsm
General average					
μ	975	3.987	0.016		
Lactations **		f_{tab}= 6,660		df_1=6	df_2=949
I	292	-0.213	0.035	3.774	0.021
II	230	-0.134	0.024	3.854	0.012
III	170	-0.076	0.015	3.911	0.011
IV	116	-0.019	0.011	3.968	0.019
V	81	0.061	0.017	4.048	0.029
VI	48	0.140	0.027	4.128	0.040
≥VII	38	0.241	0.041	4.229	0.056
ns – P>0.05		*** – P<0.05**		**** – P<0.01**	

Milk production is characterized by a regular trend of increase in the subsequent lactations, so that the minimum milk yield is in the first lactation, where the deviation from the general average was - 927.92 kg, and the maximum in the fifth with a deviation from the general average of 321.71 kg. Highly significant effect (P<0.01) of lactation on milk yield has also been established by *Trifunovic et al. (2002).*

The situation is identical with the milk fat content, since the trend of increase is present starting from the first lactation, 3.77% to 4.23% in the seventh lactation.

The lactation highly significantly (P<0.01) influenced the variability of milk fat yield. The first lactation had the greatest negative deviation from the average of -49.31 kg, and therefore the lowest yield 180.94 kg. Milk fat yield gradually increased to 250.86 kg in the sixth lactation which is marked with the highest positive deviation 20.62 kg. Approximately similar results were obtained in the research by *Trifunović et al. (2002), Petrović M.D et al. (2005),* who found highly significant effect of lactation on milk fat yield.

For the purpose of more efficient comparison of milk production, 4% fat - corrected milk is often used. The effect of lactation was highly significant (P<0.01) on the yield of 4% FCM. The minimum yield was in realized in the first lactation 4644.66 kg with a deviation from the general average of -1,110.81 kg. The yield gradually increased until the sixth lactation, which was 6185.25 kg with maximum deviation of 429.79 kg.

Table 4. General average (μ), deviation from the general average (ĉ), least square mean values (lsm) and their errors (S) and significance of studied effects on milk fat yield in complete lactation, kg

Effects	**N**	**ĉ**	**Sc**	**lsm**	**Slsm**
General average					
μ	975	230.24	4.56		
Lactations **		f_{tab}***= 16,715***		df_1***=6***	df_2***=949***
I	292	-49.31	10.07	180.94	6.12
II	230	-17.53	6.97	212.72	3.53
III	170	0.54	4.36	230.78	3.12
IV	116	9.56	3.19	239.81	5.36
V	81	16.47	4.79	246.71	8.34
VI	48	20.62	7.71	250.86	11.60
≥VII	38	19.65	11.80	249.89	15.92
ns – P>0.05		*** – P<0.05**		**** – P<0.01**	

Table 5. General average (μ), deviation from the general average (ĉ), least square mean values (lsm) and their errors (S) and significance of studied effects on yield of 4%FCM in complete lactation, kg

Effects	N	ĉ	Sc	lsm	Slsm
General average					
μ	975	5755.47	111.71		
Lactations **		f_{tab}= 17,236		df_1=6	df_2=949
I	292	-1110.81	246.78	4644.66	150.05
II	230	-355.98	170.75	5399.49	86.42
III	170	63.74	106.96	5819.21	76.56
IV	116	253.07	78.10	6008.54	131.35
V	81	375.68	117.34	6131.15	204.39
VI	48	429.79	189.09	6185.25	284.50
≥VII	38	344.51	289.19	6099.97	390.26
ns – P>0.05		*** – P<0.05**		**** – P<0.01**	

Based on results presented in the paper, it can be concluded concluded that Simmental cows included in the present study, significantly increased milk and milk fat yield to the age of approxinately 7 years, i.e. during the first five lactations. After the 7. Year of age or after the fifth lactation milk yield stagnated, and subsequently started to decline significantly to reach approximately the level of the first calving heifers at the age of 15 or 16 years.

Knowledge of the impact of external factors on the milk properties and fertility is very important with respect to their importance in achieving breeding goals and good economic results.

Table 6. General avera, least square mean values and their errors, and significance of the effect of lactation on reproductive traits

	Durat. of gestation		Service period		Calving interval	
Lactation	**LSM**	**Slsm**	**LSM**	**Slsm**	**LSM**	**Slsm**
Gen.average	286.31		110.37		398.44	
1.	284.39	0.54	112.38	4.68	398.17	4.50
2.	285.88	0.62	109.94	5.23	396.13	5.05
3.	285.93	0.61	98.65	5.94	385.91	5.96
4.	286.73	0.83	106.31	7.21	392.84	7.04
5.	285.93	0.96	111.82	7.97	397.66	8.01
6.	284.99	1.15	110.15	10.55	397.35	10.30
≥7.	285.65	1.32	113.52	11.86	400.34	11.82
F-test	$f_{tab}=1.251^{ns}$		$f_{tab}=0.935^{ns}$		$f_{tab}=0.774^{ns}$	

N.S – P >0.05; * - P < 0.05; * * - P< 0.01

Problems with fertility have become one of the most expensive factors in dairy cattle production. Selection for maternal fertility is becoming increasingly important, and requires its greater involvement in the total breeding merit/breeding value. If there are serious problems in reproduction, in addition to direct effects on milk and milk fat production, difficulties occur in the normal implementation of the herd renewal, which reflects on the cost of production (*Pantelić et al. 2009*).

The general average of least square mean values of duration of gestation corrected for the impact of lactation was 286.31 days.

Environmental factors, i.e. lactation had no significant effect on this trait (P>0.05). This can be explained by the fact that the duration of gestation is a biological constant characteristic of the particular species on which external factors have limited effect.

Average service period in studied Simmental cows was 110.37 days. Lactation had no effect (P>0.05) on the duration of service period (Table 6). This is also confirmed by research results of *Stojić et al. (1993). Pantelić et al. (2008)* have established, by the method of least squares, average service period of 115.19 days and age at first calving of 795.53 days of first calving Simmental cows kept on individual farms.

Most of the research related to the duration of the service period have indicated that its optimal duration should be up to 90 days, during which time a full involution of the genital organs is completed, and they are ready for the next conception, on the other hand, it is also the best time period from the economic point of view with regard to the natural course of lactation of cows.

Calving interval is the best indicator of cow fertility. Its duration is directly related to the duration of the service period and duration of gestation. Calving period lasted on average of 398.44 days. Lactation caused slight variations in calving period, which were not statistically significant (P>0.05).

Conclusion

Since the fastest genetic improvement through selection and improvement of production performance is through sire-son line, selection of bull dams and sires should be given the utmost attention.

Knowledge of the impact of external factors on the properties of milk and fertility is very important with respect to their importance in achieving of breeding goals and good economic results.

Selection for maternal fertility is becoming increasingly important, and requires its greater involvement in the evaluation of total merit/breeding value. If there are serious problems in reproduction, in addition to direct effects on milk and

milk fat production, difficulties arise in the normal implementation of the renewal of the herd, which reflects on the cost of production.

In order to achieve high milk and milk fat yield, satisfactory fertility, in addition to strict selection, it is necessary to provide adequate rearing conditions, especially in regard to nutrition, housing and care.

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Fenotipska varijabilnost proizvodnih osobina u populaciji krava simentalske rase

V. Pantelić, D. Ružić-Muslić, M.M. Petrović, D. Nikšić, D. Ostojić-Andrić, S. Aleksić, M. Lazarević

Rezime

Odgajivački cilj za simentalsku rasu goveda je postizanje maksimalnih genetskih vrednosti za ekonomski važne osobine, a u skladu sa ekonomskim efektima genetskog poboljšanja, koju je ova rasa postigla u razvijenim zemljama sveta.
Ovim istraživanjem su obuhvaćene 302 krave simentalske rase odabrane u kategoriju bikovskih majki na području Republike Srbije u toku jedne godine. Ispitani su uticaji paragenetskih faktora i reda laktacije na proizvodne osobine (mlečnost i plodnost). Red laktacije uticao je na pojavu visoko značajnih odstupanja od opšteg proseka ($P<0,01$) u prinosu mleka, sadržaju i prinosu mlečne masti i 4% MKM, kao i na neznatna variranja dužine bremenitosti i laktacije, međutelidbenog intervala i servis perioda, koja nisu bila statistički značajna ($P>0,05$).
Bikovske majke simentalske rase obuhvaćene ovim ispitivanjima za laktaciju u trajanju od 311,45 dana, ostvarile su proizvodnju mleka od 5.754,49kg, sa 3,98% i 230,24kg mlečne masti, odnosno 5.755,47kg 4% MKM. Prosečno trajanje bremenitosti ispitivanih grla iznosilo je 286,31 dana, servis perioda 110,37 dana, i međutelidbenog intervala 398,44 dana.

Celokupnim sagledavanjem i poznavanjem većeg broja paragenetskih faktora, kao i njihovih međusobnih interakcija na određene proizvodne osobine može se uticati na poboljšanje mlečnosti i plodnosti krava.

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MICROCLIMATE PARAMETERS ON THE CATTLE FARMS AND SOME TECHNOLOGICAL SOLUTIONS FOR ELIMINATION OF THEIR HARMFUL INFLUENCE

P. Mijić

Faculty of Agriculture in Osijek, Ul. kralja Petra Svačića 1d, 31000, Osijek, Republic of Croatia
Corresponding author: Pero.Mijic@pfos.hr
Invited paper

Abstract: Weather conditions outside the barn and microclimate in the barn have a major impact on health, animal welfare and milk production. Highly productive cows require considerable improvement of environmental conditions such as indoor and outdoor accommodation conditions. The European Union was prescribed certain guidelines and directions for the microclimate conditions that should be in the barn, but a veterinary inspection is responsible for monitoring the implementation of the law. When assessing the microclimate in the barn must take into account various aspects, such as: breed, age, productions, housing, and the duration and intensity of the effect of certain factors of microclimate in the barn. The most important parameters of the microclimate in the barn are: air temperature in the barn and temperature of the surface of the certain parts on the barn, the relative humidity in the barn, velocity of wind, light and concentrations of harmful gases.To ensure the welfare of animals and therefore positively influence on the increasing of milk production it is necessary to take appropriate measures to contribute to the cows in thermoregulation and prevent the occurrence of heat stress. This can be achieved by placing fans and sprinklers to cool cows with water, shades, feeding time, etc. For some solutions, the investments are necessary but for a long period of time they are profitable.

Key words: microclimate, barn, cattle, milk production, heat stress

Introduction

Weather and climate conditions in the wider and microclimate in the barn in the strict sense have a major impact on animal health, welfare and the milk production *(Kadzere et al., 2002; Nardone et al., 2006; Mijić et al., 2007; Mačuhova et al., 2008; Brouček et al ., 2009).* Appearance of the heat stress has a negative influence on the milk production, but besides that has a negative impact on the animal welfare *(Young, 1993; Jacobsen, 1996).* Highly productive cows

require considerable improvement of environmental conditions such as indoor and outdoor accommodation conditions, which makes the microclimate a very important link that affects the welfare of animals through certain holding system (*Bobić et al., 2011*). During high temperatures the cows which are grazing will seek shade, and cows which are in the barn are looking for the place with lowest temperature *(Brouček, 1997)*. Should take into account the fact that the local indigenous breeds of cattle better tolerates stressful climatic conditions than a conventional breeds (*Mijić et al., 2009a*). Besides the impact of the microclimate parameters on the animals, also has an impact on the people who work on the farm because they also spending some time in the barn (*Mijić and Bobić, 2009b*).

Certain guidelines and directions for the microclimate conditions that should be in the barn, which are described in this paper, are prescribing the authorities from European Union, but a veterinary inspection is responsible for monitoring the implementation of the law *(Scientific Veterinary Committee, 1997)*. However, there are no specific regulations and recommendations for the precise sequence of measurements of each microclimate parameter in the barn. Checking the climate in the barn is problematic because of the relevance, comparability and legal certainty of the results obtained by measurement. The aim of this study was to show the importance of the microclimate in the barn in modern cattle production, and suggest the most important parameters that affect the microclimate and opportunities for her improving.

The parameters of the climate in the barn and their measurement

When assessing the microclimate in the barn must take into account various aspects, such as: breed, age, productions, housing, and the duration and intensity of the effect of certain factors of microclimate in the barn. Also, it is important the farms elevation and the region of the cattle breeding, respectively (*Mijić et al., 2010*). Some parameters cannot be observed separately, but must take into account their mutual interactions. Besides of that, it is important a way and design of ventilation, window size and other technical equipment which is located in the barn, as well as a number of animals.

The most important parameters of the microclimate in the barn are: air temperature in the barn and temperature of the surface of the certain parts on the barn, the relative humidity in the barn, velocity of wind, light and concentrations of harmful gases.

Measurement of microclimate in the barn is taken in an area where animals are kept. When is the measuring it is important to consider yearly age, time of measurement, the weather conditions and the position of the measurement point in the barn, because that all has an effect on the results.

Measurements which are made over a longer period of time and at different times are more accurate indicators of actual conditions in the barn than a single measurement. Continuous measurements are those which are performed using certain devices ("Data loggers") fitted to farms with independent measurements. Compared with the single measurements, continuous measurements allow us a more realistic view of climate in the barn, because the measurement is carried out over a longer period of time. However, continuous measurements have their shortcomings; the devices must often set slightly above the zone in which the animals are to be protected from damage. In determining the microclimate in the barn the must are taken into account and a microclimate conditions outside the barn. Because of that it is necessary to measure: air temperature, relative humidity, wind velocity and direction of the wind from outside of the barn (*Mijić and Bobić, 2012*).

Air temperature in the barn

Depending on authors, the optimal temperature for dairy cows ranging from 4 to 25 °C *(Roenfeldt, 1998, Heidenreich et al., 2004)*. Experience has shown that cows have the largest capability for production of milk, if the temperatures are in the optimal range. They apply to just the area of the environment in which the cows linger longer period of time, although the cows have the ability to adapt to a significantly higher temperature range.

High milk production leads to the formation and release of large amounts of metabolic heat due to the process of digestion and decomposition of large amounts of food consumed. Increase in temperature above the optimum value, cows lose the ability for the adequate cooling and entering the heat stress *(Bligh, 1973; Gantner et al., 2011)*. However, the cows had significantly greater tolerance to low *(Hemsworth et al., 1995; Wassmuth, 1999),* but they have to high temperatures *(Legates et al., 1991; Lacetera et al., 2002)*.

Highly productive cows are much more susceptible to heat stress compared to low productive cows *(Kadzere et al., 2002)*. That supports the research of *Tapki and Sahin (2006)* who observed a significant difference ($p < 0.001$) between high production and low production cows with increasing temperature. Percentage feeding (31.7 to 17.4%) and rumination (18.1 to 14.6%) is reduced, while the extended time of standing (25.0 to 38.4%) and drinking water (5.2 - 7.4%) in high-producing cows. There is a difference in sensitivity to elevated temperatures of the different stages of lactation, so for example, cows that are in earlier stages are more sensitive and have a greater decline in production during exposure to high temperatures compared to cows in the later stages of lactation *(Igono and Johnson, 1990)*.

During the high summer temperatures cows reduce food intake *(West, 2003),* thereby protecting their body from overheating, resulting in a decrease in milk production. Besides of the air temperature in the barn, essential is the temperature of the individual parts of a barn such as the temperature of the roof. The surface temperature and also the material at which the cow lying is also of great importance. In the winter, can lead to hypothermia due to heat loss and in the summer can cause problems with thermoregulation due to lower thermal conductivity, especially on deep litter.

Relative humidity in the barn

Bockisch et al. (1999) suggest that the humidity and temperature have a significant impact on the potential possibility of infection and fertility in cows. To get the most accurate information is necessary to perform continuous measurements of the relevant intervals, because the current measurements can serve only for orientation purposes. *Nauheimer and Weniger (1986)* suggest that the negative effects of high temperature on milk production and energy intake in cows in early lactation (30 ° C at a relative humidity of 50% compared to 15 ° C and relative humidity of 70%). *Brunsch et al. (1996)* suggested that the optimum humidity for cows is 60 to 80%.

In the open type barns the relative humidity and temperature inside the barn were largely the same as external conditions. Therefore, it is a necessary to have fans and sprinkles of water in the barn during high summer temperatures, in order to allow cows to better cooling and housing conditions. These technical procedures will certainly have a positive effect on animals, and therefore the production of milk.

Velocity of the wind

Ventilation has the task of creating an optimal climate in the barn, which is a prerequisite for health, welfare and high milk production. In addition, the ventilation has the role of protecting the building from the damage *(Mačuhová et al., 2008).* The properly constructed barns and implementation of ventilation can largely meet the ventilation function. However, during the high temperature in the summer is necessary to take additional measures to enhance the natural flow of air in the barn because of high production cows come to the limit of their capacity for thermoregulation. *Heidenreich (2009)* states that the greatest effect of cooling is achieved with air velocity of 2.5 m/s. There are many ways to increase the speed of air flow in the barn. In the open type barns prerequisite for good air circulation is choice of location for the construction and proper management with side curtains in the barn. However, with all these the fans are still necessary to achieve better air flow in the barn.

Illumination

The light is of great importance to cattle, because affects on vision of cattle while are staying in the barn or in the pasture, affects the growth, production and welfare. Important features of light are: intensity and duration of light, the spectral composition of light and technical parameters such as blinking lights. For newly built stables there are guidelines of the European Union, according to which the barn should be open for entry of natural light, the area of which shall be at 3-5% of the total floor area stalls. When there is not enough natural light, an artificial lighting is needed. The minimal intensity of light is an 80 lux, for a period longer than 10 hours. The regime of the light should be determined according to the natural rhythm of day-night (including the phase of twilight), where dark phase or phase of night is should be a minimum of 8 hours *(Praktische Tierhygiene, 2010)*.

Table 1. Needs of cattle for illumination *(LfL, 2012)*

	Duration of illumination (h)	Intensity of illumination (lux)
Calf and cows in lactation	10-16	100-200
Cows in dry period	8	100-200

Concentration of harmful gases

Harmful gases in the barn, can be only partially discovered with odor. Paralysis of the fragrant senses are occurs with a higher concentrations of H_2S ($\geq$ 200 ppm). Upon entering in the barn are feeling a stifling air, chaffing of eyes and respiratory mucosa. These are the first indicators of bad microclimate in the barn. When the ventilation in the barn are insufficient or inadequate, are increased the concentration of harmful gases. In the research of climate in the barn as relevant gases is measured: CO_2, NH_3 and H_2S. CO_2 can also be used to assess the effectiveness of the ventilation system. Unlike CO_2, the NH_3 and H_2S can be harmful already in low concentrations (NH_3, 30 - 40 ppm). Since the concentration of gases in the barn is constantly changing, it is necessary to perform continuous measurements, for at least seven days. This measurement gives a more realistic picture of the actual gas concentrations in the barn.

Table 2. The limit values for harmful gases in the barn

Source	CO_2 (ppm)	NH_3 (ppm)	H_2S (ppm)
Praktische Tierhygiene (2010)	3000	20	5
Scientific Veterinary Committee (1997)	3000	10	0,5 (briefly during removal of the manure 5 ppm)

Technological solutions to combat unfavorable microclimate conditions and consequences of heat stress

Since it took place the great climatic changes over the last few decades, which has a strong impact on today's selection productive cows, should be given a few words about heat stress and possible technological solutions to this problem on modern dairy farms.

During high temperature in the open type barns ventilation is not sufficient to completely cooling of cows. To ensure the welfare of animals, and therefore get a positive influence on the increasing of milk production, it is necessary to take appropriate measures to contribute to the cows in thermoregulation. This is achieved by heat exchange between cows and their environment: conduction, convection, radiation, evaporation (*Kadzere et al., 2002)*. Below are some measures to protect cows from the occurrence of heat stress by some authors *(Flamenbaum et al., 1986, Pennington and VanDevender, 1914; Blackshaw and Blackshaw, 1994; Silanikove, 2000).*

Shade

Shade reduces heat stress by decreasing exposure to solar radiation. Ideal barns with free grazing should be oriented from east to west to allow the maximum amount of shadow from the roof. During periods of extreme heat stress (high temperatures, high humidity) night time temperatures do not provide adequate relief from heat stress. During such times, access to pasture at night should be allowed to cows because cows release more heat through radiation to the night sky than the roof of the barn.

Ventilation

Proper ventilation is essential to cooling of cows. A good ventilation system should provide a complete air exchange per minute. This is usually achieved by using fans. Every fan should allow air flow of at least 0.3 m^3/min. Fan must be placed at an angle so that the air flows over the back of cows. It is also important to allow natural ventilation in barns. Side vents and openings in the roof must be of optimal size (at least 0.3 meters wide, plus 5 cm to 3 m in height stops). Heat stress is highly expressed in the waiting area, and it is extremely important to set the fans there and allowed to cool cows while waiting for milking.

Water

Heat stress greatly increases water intake in cows. For example, an increase in temperature from 30 to 35° C increases water intake in lactating dairy cows by over 50 percent. It is important to allow plenty of fresh, clean and cool water. Ideally cows should not walk more than 15 meters to get to the water, and in this way should not pass routes that do not have a shadow and should not stand

in, direct sunlight to drink. It is also a good strategy to provide fresh, clean water in the exit area of the milking parlor, since the water consumption is usually highest right after milking.

Supplemental cooling

Supplemental cooling using sprinklers are another strategy. Spraying cattle with water allows faster cooling of cows and even better performance is achieved by a combination of the fans. Since the waiting area most critical place, it is desirable that particular set sprinklers, but always with a combination of the fan. Otherwise, you can create the opposite effect and result with saunas effect.

Feeding time

Heat stress is greatest when temperatures are highest. Feeding at this time leads to reduced food intake and increases the temperature of the food and spoilage. It is recommended that the gradual replacement feeding times especially high dairy cows in cooler parts of the day (early morning or late evening). You often raking up of food and extract for food spoilage increases dry matter intake.

Ration formulation

Heat stress causes a reduction in the dry matter intake. It is necessary to increase the energy in the diet, and not to increase metabolic heat due to fermentation. This can usually be achieved by adding inert fat in the rumen and "bypass protein". Potassium levels should also be compensated because it is lost with sweating of cows.

Stocking density

Many farmers, overloaded, their stalls with too many cows. Under conditions of high heat stress overload with cows just aggravated the situation. Should take into account the reducing stocking densities to 100 percent and provide approximately 70cm of feed bunk space per cow to encourage dry matter intake.

Conclusion

In order to achieve better production results in modern cattle production, we must ensure optimal conditions for the animals. The key role in this has barn and microclimate conditions. Cows with high milk production have increased production of body temperature due to which at high ambient temperatures often suffer from heat stress. During high temperature in open type barns natural ventilation is not sufficient to completely cooling of cows. To ensure the welfare of animals and therefore positively influence on the increasing of milk production it is necessary to take appropriate measures to contribute to the cows in

thermoregulation. At the same time, the investments are necessary but for a long period of time they are more profitably.

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Mikroklimatski parametri na farmi goveda i neka tehnološka rešenja za uklanjanje njihovog štetnog uticaja

P. Mijić

Rezime

Vremenski uslovi izvan ambara i mikroklima u štali imaju veliki uticaj na zdravlje, dobrobit životinja i proizvodnju mleka. Visoko produktivne krave zahtevaju značajno unapređenje stanja životne sredine, kao što su unutrašnji i spoljni uslovi smeštaja. Evropska unija je propisala određene smernice i uputstva za mikroklimatske uslove koji treba da budu u štali, ali je veterinarska inspekcija odgovorna za praćenje primene zakona. Prilikom procenjivanja mikroklime u štali moraju se uzeti u obzir različiti aspekti, kao što su: rasa, starost, produkcija, stanovanje, kao i trajanje i intenzitet uticaja pojedinih faktora mikroklime u štali. Najvažniji parametri mikroklime u štali su: temperatura vazduha u štali i temperatura na površini određenih delova u štali, relativna vlažnost vazduha u štali, brzina vetra, svetlost i koncentracija štetnih gasova.Da bi se osigurala dobrobit životinja i samim tim pozitivno uticalo na povećanje proizvodnje mleka potrebno je preduzeti odgovarajuće mere da se pomogne kravama u termoregulaciji i spreči pojava toplotnog stresa. Ovo se može postići postavljanjem ventilatora i prskalice za hlađenje krava sa vodom, roletne, vreme za jelo, itd. Za neka rešenja, investicije su neophodne ali dugoročno su profitabilne.

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EFFECT OF FIXED AND CONTINUOUS NON-GENETIC FACTORS ON LENGTH OF SERVICE PERIOD IN SIMMENTAL COWS

M.D. Petrović[1], M.M. Petrović[2], V. Bogdanović[3], S. Bogosavljević-Bošković[1], R. Đedović[3], S. Rakonjac[1]

[1]Faculty of Agronomy, Cara Dušana 34, 32000, Čačak, Republic of Serbia
[2]Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080, Belgrade-Zemun, Republic of Serbia
[3]Faculty of Agriculture, Nemanjina 6, 11080, Belgrade-Zemun, Republic of Serbia
Corresponding author: milunp@kg.ac.rs
Invited paper

Abstract: Cattle reproduction is a highly important field and complex stage of production with large reserves of milk, meat, breeding cattle and by-products. In scientific literature, fertility is generally assessed through age at first conception, gestation length, length of service period, calving interval, and calf birth weight. Determination of the effect of particular non-genetic factors on the above traits is a vital step in cattle breeding and reproduction. Length of service period is largely governed by the effect of non-genetic factors, viz. fixed effects generally including the effect of breeding region, season of birth, calving season, year of birth, calf sex and their interactions, and continuous or regression effects including age at first conception or age at calving. The effect of fixed and continuous non-genetic factors on length of service period was analysed in 245 Simmental cows (907 service period) in three breeding regions, with the effect of season of birth as a fixed factor and cow age at first conception as a continuous factor being highly significant ($P < 0.01$), and that of season of calving being significant ($P < 0.05$). The effect of breeding region, year of birth, parity group or age and calf sex was statistically non-significant ($P>0.05$). Therefore, the coefficient of determination showing the level of variation in service period as explained by the effect of non-genetic factors fitted in the model was low (0.078).

Key words: Simmental breed, service period, fixed non-genetic effects, continuous non-genetic effects, coefficient of determination

Introduction

Cattle reproduction is a very important research field and a highly complex stage of cattle production securing reserves of milk, meat, breeding herds and by-products.

Fertility traits of great importance, which is expressed in the normal course of the production cycle. One of their most important specificity is pronounced variability and low heritability (*Novaković et al., 2011*).

At the current level of development of agricultural production and animal science, the issue of milk and meat production is considered less serious than that of cattle reproduction management. Cattle reproduction physiology is a complex field affected by a range of endogenic and exogenic factors more intensely than any other stage of cattle production. It is noted that there is a cow productivity limit beyond which normal fertility cannot be maintained. In their study on the effect of production levels on length of service period, *Hakana et al. (1995)* reported very significant differences ($P<0.01$) in length of service period between cows with high and low production levels (106.5 and 82.5 days, respectively). *Heushow (1993)* suggests that full economic returns from a herd can be achieved if oestrus occurs within 60 days following parturition in 85% of cows, if 70% of cows conceive after first insemination and if 60% of the conceived cows calve. The insemination index should be 1.6, the percentage of problem cows in a herd should be below 10% and the proportion of cows showing silent oestrus below 15%. Service period should be shorter than 100 days, as stated by the said author.

In their study on cattle fertility, *Trifunović et al. (1990)* provided a classification of the parameter, with the service period of 70 days, 71-90, 91-110 and over 110 days being classified as excellent, very good, satisfactory and unsatisfactory, respectively. Unfortunately, the unsatisfactory service period of over 110 days is quite common in practice, as reported by most authors.

Schmitz (1996a, 1996b, 1997 and 1998) report the average service period of 95-97, 89-95 and 93-99 days in Simmental cows with 14%, 14-74% and 75-100% Red and White Holstein (RWH) inheritance, respectively.

Petrović D.M. et al. (2009) reported the average service period of 137, 146 and 179 days in 2805 Simmental cows kept in three breeding regions under different housing systems and at different altitudes. Service period was longest in cows housed on privately owned upland mini farms that had no adequate rearing conditions available due to land fragmentation, low financial resources and low level of knowledge regarding animal husbandry practices. In contrast, the other two state-owned farms provided better conditions and, hence, ensured a considerably shorter service period, although longer than optimum (137 and 146 days, respectively).

Pichler (2004) reports the service period of 90-95 days for the Simmental breed as a breeding programme target in the Czech Republic.

Research conducted by *Pantelić et al. (2005)* on the effect of non-genetic factors on reproductive traits of Simmental bull dams resulted in the overall least squares mean for length of service period of 108.98 days.

The same author, *Pantelić et al. (2008),* using the method of least squares, have established very significant effect of region on the service period, which amounted to 115. 19 days. Studying the production and reproduction traits of Simmental and Holstein Friesian cows of the Semberia, *Budimir et al.(2011*) point out that the service period after the first three lactations in Simmental cows was 163.97, 136.85 i 126.43 days.

Material and Methods

Effect of non-genetic environmental factors on length of service period was evaluated in 245 Simmental cows with a total of 907 service period housed on privately-owned mini farms under tie-stall system in the Municipality of Čačak (n=67 cows and n=297 service period), Zlatibor (n=123 cows and n=439 service period) and Rudno region (n=51 cows and n=171 service period). The Municipality of Čačak is a lowland, whereas Zlatibor and Rudno regions are upland areas.
The effect of the following systematic factors on service period length was evaluated:

- ***Breeding region (farm).*** Three locations employing a tie-stall housing system were covered by the study, two in the upland region (II – mini dairy farms on Mt. Zlatibor and III – on Rudno highlands) and one in the lowlands (I – mini farms in the Municipality of Čačak).
- ***Parity groups.*** To reduce variability induced by the decrease in the number of cows across parity groups, the effect of five groups of parity (group I (1st parity), group II (2nd parity), group III (3rd parity), group IV (4th parity), group V (5th and above)) rather than the effect of each individual parity, was evaluated.
- ***Calving season i.e. the onset of lactation.*** The effect of four seasons, including I – spring season (March, April, May), II – summer season (June, July, August), III – autumn season (September, October, November) and IV – winter season (December, January, February) was analysed.
- ***Season of birth.*** Effects of four seasons, viz. I-spring season (March, April, May), II-summer season (June, July, August), III-autumn season (September, October, November), and IV-winter season (December, January, February) were evaluated.
- ***Year of birth.*** Ten years of birth i.e. cows born during 1998-2007 were covered by the experiment.
- *Calf sex:* I-male calves, II – female calves.

- ***Year of birth x season of birth interaction*** (10 years of birth (1998-2007) x 4 seasons of birth).
- ***Age at first conception.***

The analysis of the effect of the above systematic environmental factors was conducted using a general linear model enabling a simultaneous analysis of a number of different effects, irrespective of their being categorical or continuous ones. The general linear model involved the use of the least squares method for the evaluation of the effects and testing of the hypotheses, according to the following model:

$$\mathbf{y}_{ijklmn} = \mu + \mathbf{R}_i + \mathbf{P}_j + \mathbf{Sc}_k + \mathbf{Sb}_l + \mathbf{Yb}_m + \mathbf{S}_n + \mathbf{YbSb}_{ml} + \mathbf{b}_1(\mathbf{x}_1 - \bar{x}_1) + \mathbf{e}_{ijklmn},$$ where

$\mathbf{y}_{ijklmn}$ - individual cow of the i-th rearing area, j-th parity group, k-th season of calving, l-th season of birth, m-th year of birth and n-th sex of calf),
μ - overall population mean under identical distribution of all classes of effects (R, P, Sc, Sb, Yb, S, YbSb),
$\mathbf{R}_i$ - fixed effect of the i-th rearing area (1-3),
$\mathbf{P}_j$ - fixed effect of the j-th parity group (1-5),
$\mathbf{Sc}_k$- fixed effect of the k-th season of calving (1-4),
$\mathbf{Sb}_l$ – fixed effect of l- th season of birth, (1-4),
$\mathbf{Yb}_m$ – fixed effect of m-th year of birth (1-10),
$\mathbf{S}_n$ -fixed effect of the n-th sex of calf (1-2),
$\mathbf{YbSb}_{ml}$ – fixed effect of the m-th year of birth x l-th season of birth (1-40),
$\mathbf{b}_1$ - linear regression coefficient of the effect of age at first conception and
$\mathbf{e}_{ijklmn}$ - other non-determined effects

Further analysis of service period shows results of the analysis of variance using the above model, i.e. significance of factors, as well as the coefficient of determination (R^2), denoting the remainder and the model variance divided by 100, respectively.

Results and Discussion

The effect of systematic environmental factors on service period was analysed by calculating the least squares means (LSM) and standard errors of the means (SE_{LSM}). The results of the analysis are given in Table 1.

Table 1. Least squares means, standard errors of the means and significance of the effect of systematic factors and age at first conception on service period *(SP)*

Systematic effects	Service period - SP (days)		
	N	LSM	SE_{LSM}
Total	*907*	*104.44*	*2.132*
Farm			
I	297	104.03	3.423
II	439	103.50	3.025
III	171	107.55	5.703
F_{exp}		0.218^{ns}	
Parity groups			
I (1)	-	-	-
II (2)	248	111.98	4.481
III (3)	248	101.49	4.006
IV (4)	248	100.22	3.786
V (5 and remain.)	163	103.86	4.864
F_{exp}		1.783^{ns}	
Calving season			
I	250	97.84	3.162
II	223	106.90	4.543
III	186	101.81	4.417
IV	248	110.84	4.776
F_{exp}		2.676*	
Season of birth			
I	251	102.10	3.902
II	256	98.89	3.821
III	160	107.22	4.708
IV	240	110.94	4.656
F_{exp}		8.332^{**}	
Calf sex			
I	448	102.62	2.786
II	459	106.20	3.220
F_{exp}		1.376^{ns}	
Year of birth			
F_{exp}		1.237^{ns}	
Year of birth x season of birth			
F_{exp}		1.434^{ns}	
Age at first conception			
F_{exp}		7.196^{**}	
Coefficient of determination – R^2		$0,078^{**}$	

*N.S. - P > 0.05; * - P < 0.05; ** - P < 0.01; *** - P < 0.001;*

An average length of service period was considerably longer than that ensuring the production of one calf per year, set as a goal of milk and meat production in cattle breeding. The prolonged service period indicates the unfavourable effect of fixed non-genetic factors.

The effect of farm on length of service period was non-significant (P>0.05), suggesting similar rearing conditions and technology. A non-significant effect of breeding region on length of service period was observed by *Đurđević (2001),* whereas significant and very highly significant effects were reported in a considerably larger number of studies (*Skalicki et al., 1991; Pantelić et al., 2005; Petrović D.M. et al., 2009)*.

Similarly to breeding region, the effects of parity group, calf sex, year of birth and the year of birth x season of birth interaction had no significant effect on fertility rate (P>0.05).The effect of parity order on service period was found to be either non-significant (P>0.05), as reported by *Đurđević (2001), Pantelić et al. (2005)* and *Petrović D.M.i sar. (2009),* or significant (P<0.05), very significant (P<0.01) and very highly significant (P<0.001) in the studies by *Caput et al. (1991), Perišić (1998)* and *Petrović D.M. (2000).* When analysing the effect of birth type, calf sex and birth type x calf sex interaction on reproductive traits in Simmental cows, *Petrović D.M. (2000) and Petrović D.M. et al. (2009)* found no significant effect of calf sex on length of service period.

The effects of season of birth, season of calving and onset of lactation as non-genetic factors on length of service period are reflected through different climate and diet conditions throughout the year, and have been the subject of a substantial number of studies.

Service period was statistically significantly (P<0.05) affected by calving season. Spring calving cows had a significantly shorter service period (97.84 days). A significant effect of calving season on service period was reported by *Đurđević (2001)* whereas a non-significant effect (P>0.05) of calving season on fertility rate was observed by *Perišić (1998), Petrović D.M. (2000) and Petrović D.M. et al. (2009).*
Length of service period was affected by season of birth. Spring- and summer-born cows had a highly significantly (P<0.01) shorter service period compared to winter- and autumn-born cows. In their study on the effect of fixed non-genetic factors on fertility traits in Simmental cows, *Petrović D.M. et al. (2009)* found fertility rate to be very highly significantly affected by the season of birth x year of birth interaction.
The regression analysis used suggested that the effect of age at first conception on service period was statistically highly significant (P<0. 01). *Perišić (1998)* and *Petrović D.M. (2009)* confirmed the highly significant (P<0.01) effect of age at first conception as the fixed factor on service period, whereas *Petrović D.M. (2000)* reported non-significant regression effects (P>0.05).

Given the non-significant effects (P>0.05) of breeding region, year of birth, parity group i.e. age, and calf sex on fertility rate, the coefficient of determination (R^2), showing the level of variation in service period as explained by the effect of non-genetic factors fitted in the model, was moderately low - 0.078 i.e. 7.8%. The low value for the coefficient of determination clearly suggests that service period was also affected by a range of genetic and non-genetic factors not included in the model. A somewhat lower coefficient of determination for service period (0.036 i.e. 3.6 % and 0.050 i.e. 5.0%) was reported by *Petrović D. M. (2000 and 2009)* in his study on the effect of systematic factors on reproductive traits.

Conclusion

The effect of fixed and continuous non-genetic factors on length of service period was analysed in 245 Simmental cows (907 calving interval) in three breeding regions, with the effect of season of birth as a fixed factor and cow age at first conception as a continuous factor being highly significant ($P < 0.01$), and that of season of service period significant ($P < 0.05$).

The effect of breeding region, year of birth, parity group or age and calf sex was statistically non-significant ($P > 0.05$). Therefore, the coefficient of determination showing the level of variation in service period as explained by the effect of non-genetic factors fitted in the model was low (0.078 i.e. 7.8 %).

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Uticaj fiksnih i kontinuiranih negenetskih faktora na trajanje servis perioda kod krava simentalske rase

M.D. Petrović, M.M. Petrović, V. Bogdanović, S. Bogosavljević-Bošković, R. Đedović, S. Rakonjac

Rezime

Reprodukcija goveda predstavlja veoma značajnu oblast i vrlo složenu fazu proizvodnje u kojoj se nalaze velike rezerve mleka, mesa, priplodnih grla i sporednih proizvoda.

U literaturi plodnost se najčešće posmatra preko uzrasta pri prvoj oplodnji, trajanja bremenitosti, servis perioda, intervala između telenja i mase teladi pri rođenju.

Determinisanje uticaja pojedinih sistematskih faktora na pomenute osobine od velike je važnosti u procesu odgajivanja i reprodukcije goveda.

Dužina servis perioda definisana je najvećim delom dejstvom paragenetskih faktora, od kojih kao fiksni uticaji najčešće se pominju uticaj odgajivačkog područja, sezone rođenja i telenja, godine rođenja, pola teladi i njihove interakcije, a od kontinuelnih ili regresijskih uzrast krava pri prvoj oplodnji ili telenju.

Analiza uticaja fiksnih i kontinuiranih negenetskih faktora na dužinu odnosno trajanje servis perioda izvršena je kod 245 krava simentalske rase (907 servis perioda) raspoređenih na tri odgajivačka područja pri čemu je uticaj sezone rođenja kao fiksnog i uzrasta krava pri prvoj oplodnji kao kontinuiranog faktora bio visoko značajan ($P<0.01$) dok je uticaj sezone telenja na dužinu servis perioda bio značajan ($P<0.05$). Uticaj odgajivačkog područja, godine rođenja, grupe partusa odnosno starosti kao i pola teladi nije bio statistički značajan ($P>0.05$), pa je shodno tome i koeficijenat determinacije, koji ukazuje na nivo objašnjenosti variranja servis perioda dejstvom negenetskih faktora obuhvaćenih primenjenim modelom nizak i iznosi svega 0.078.

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EVALUATION THE METABOLIC STATUS OF EARLY AND MID LACTATION DAIRY COWS THROUGH CHANGES IN BLOOD BIOCHEMICAL INDICATORS

Z. Ilić[1], R. Đoković [2], V. Kurčubić[2], M.P. Petrović[3], V. Caro-Petrović [3], J. Stojković[1], B. Ristanović[1]

[1]Faculty of Agriculture, University of Priština, Kopanička bb. 38219, Lešak, Republic of Serbia
[2]Faculty of Agronomy, Čačak, University of Kragujevac, Cara Dušana 34, 32000 Čačak, Republic of Serbia
[3]Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080, Belgrade-Zemun, Republic of Serbia
Corresponding author: zoran.ilic@pr.ac.rs
Invited paper

Abstract: The objective of the present study was to evaluate the metabolic status of early and mid-lactation Simmental dairy cows through changes in blood biochemical indicators. Fifteen early lactation cows and 15 mid lactation cows were chosen for the analysis. Blood samples were collected to measure beta-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), triglycerides (TG), glucose and the activity of aspartate transaminase (AST). Early lactation cows had significantly higher ($P<0.05$) values of blood BHB and NEFA, and lower glycemia ($P<0.05$) and TG ($P>0.05$) values compared to mid lactation cows. High lipomobilization (NEFA > 0.4 mmol/l) and subclinical ketosis (BHB > 1.2 mmol/l) were detected in 6 (40%) and 14 (94.4%) early lactation cows, respectively, and in none of the mid lactation cows. AST activities above 100 IU/l were detected in 2 early lactation cows and none of the mid lactation cows. TG concentrations below 0.12 mmol/l were found in 7 (44%) early lactation cows and 2 (13.3%) mid lactation cows. Glucose levels were below 2.5 mmol/l in 10 (66.6%) early lactation cows and 5 (33.3%) mid lactation cows. Blood serum values for glucose, TG, BHB, NEFA and AST showed that early lactation cows suffered from metabolic disturbances, which were associated with ketosis, and some degree of hepatic lesions, probably due to fat infiltration. Suggest that they can serve as useful indicators of the metabolic status of dairy cows during lactation.

Key words: blood indicators, subclinical ketosis, hepatic lipidosis, dairy cows

Introduction

Production diseases i.e. diseases associated with improper nutrition or management are common in dairy cows. Dairy cows suffer from negative energy

balance (NEB) during the first week of lactation due to energy expenditure associated with milk production and limited feed intake, resulting in NEB, a high mobilization of lipids from body fat reserves, and hypoglycaemia *(Veenhuizen et al., 1991; Drackley, 1999; Bobe et al., 2004; Đoković et al., 2007, 2011)*. Nutrition, age, heredity, body condition score (BCS), management and energy imbalance as various risk factors are possible causes of NEB, periparturient fatty liver and ketosis *(Drackley, 1999; Bobe et al., 2004)*. Clinical ketosis in dairy cows usually occurs between the second and seventh week of lactation. Nevertheless, most of cows in this stage of lactation may suffer a subclinical form of ketosis defined as increased blood ketone bodies without any other symptoms but accompanied by considerable decrease in milk yield and susceptibility other diseases *(Duffield et al., 1997)*. The main blood indicators of lipomobilization in ruminants are BHB, the most important and abundant ketone body, and NEFA *(Civelek et al., 2011; González et. al., 2011)*. NEFA are preferentially and greatly accumulated as TG in the liver, primarily because of a decrease in the very low density lipoproteins (VLDL) synthesis by hepatocytes *(Herdt et al., 1983; Sevinc et al., 2003)*. However, when steatosis occurs, endogenous liver synthesis decreases, leading to a reduction in blood glucose, total proteins, albumins and globulins, cholesterol, TG and urea. *(Herdt et al., 1983; Veenhuizen et al., 1991; Sevinc et al., 2003; Đoković et al., 2007, 2011)*. Fatty liver infiltration and hepatocyte degeneration involve cell membrane damage and hepatocyte destruction coupled with the release of cytoplasm enzymes (AST, GGT, LDH) and marked increases in the circulating activities *(Pechova et al., 1997; Lubojacka et al., 2005; González et. al., 2011)*. Diagnosing liver lipidosis and susceptibility to ketosis in dairy cows may include liver biopsy or echography, but a less invasive and more economical analytical method may be the measurement of blood biochemical indicators *(Baird, 1982, Bobe et al., 2004)*. Based on blood biochemical indicators, ketosis in cows may be diagnosed when the following values match both the clinical signs (BHB > 1.2 mmol/l, glucose < 2.5 mmol/l, and TG < 0.12 mmol/l) and blood values of NEFA > 0.7 mmol/l and AST activity above 100 IU/l, which is indicative of hepatic lipidosis *(Sevinc et al., 1998; Oetzel, 2004; González et al., 2011)*. The objective of the present experiment was to evaluate the metabolic status of early and mid-lactation cows through changes in blood biochemical indicators.

Material and methods

Animals: This experiment was carried out in January 2012 in a dairy herd (119 Simmental cows) suffering from several metabolic and reproductive disorders (Farm: Ćurćić, Mrsać, Kraljevo, Central Serbia). The cows were mid-yielding with a preceding lactation of about 6.500 l (early lactation cows: 23.5 ± 4 l /day/ cow,

mid lactation cows: 28.5 ± 6 l / day / cow, calculated on the basis of milk yield in the previous lactation). Two groups of clinically healthy cows were chosen from the herd. One group consisted of early lactation cows (n = 15) in the first month of lactation (16.1± 9.3 days), and the second group included mid lactation cows (n=15) between 3 to 5 months of lactation (114.81 ± 28.5 days). Body condition scores (BCS) were recorded by the same observer using the 1~5 scale according to Ferguson et al. (1994), with 1 meaning = too thin and 5 = too fat. BCS were 3.42 ± 0.55 and 3.27 ± 0.42 in early lactation and mid lactation cows. The experimental cows were kept in tie-stall barns. Diet and the housing facilities were adapted to research purposes, with diet suited to the energy required by early and mid-lactation cows. Early lactation cows were fed a diet consisting of 7 kg lucerne hay, 20 kg maize silage (30% Dry Matter, DM), 5 kg concentrate (18% crude proteins, CP). Mid lactation cows received a diet consisting of 5 kg hay, 7 kg lucerne hay, 30 kg maize silage (30% DM), 8 kg concentrate (18% CP). Dietary nutrient contents for dairy cows in early and mid-lactation are given in Table 1.

Table 1: Nutrient contents in daily ration for early lactation and mid lactation dairy cows.

	Early lactation cows	Mid lactation cows
Dry Matter (DM) (kg)	16.05	24.82
Net Energy of Lactation (NEL) (MJ)	87.15	130.23
Crude Protein (CP) (% of DM)	13.58	13.38
Rumen undegradable protein (RUP) (% of CP)	35.91	28.33
Fat (% of DM)	3.09	3.14
Fiber (% of DM)	23.26	24.33

Biochemical analysis: Blood samples were collected at 10:00 h or 4 to 6 hours after milking and feeding, by puncture of the jugular vein into sterile disposable test tubes, without anticoagulant. After clotting for 3 hours at 4°C and centrifugation (1500g, 10 minutes, 4°C), sera were carefully harvested and stored at -20°C until analysis. Blood samples collected on fluoride were immediately centrifuged in the same manner and plasmas were assessed for glucose concentrations. The following biochemical blood components were measured at Kvarklab Biochemical Laboratory (Kragujevac, Serbia) by different colorimetric techniques using spectrophotometers (Cobas Mira and Gilford Stasar): beta-hydroxybutyrate (BHB) and non-esterified fatty acid (NEFA) levels were measured by Randox (United Kingdom) kit, aspartate transaminase (AST), glucose by Human (Germany) kit, and triglyceride (TG) by Elitech (France) kit.

The statistical analysis of the obtained data was carried out by ANOVA-procedure (Statgraphic Centurion, Statpoint Technologies Inc.Warrenton, Va, Virginia, USA). The analysis of variance and LSD test were used to evaluate the

probability of the significance of the statistical differences between mean parameter values in each group and the Pearson test was performed for evidencing significant correlations. Differences were considered as significant when P values were below 0.05 or 0.01.

Results and Discussion

Results on blood biochemical indicators for both groups of cows are shown in Table 2.

Table 2.Blood indicators in early and mid lactation dairy cows (n=15 in each group). Results are expressed as mean ± standard deviation (SD).NS: non-significant

	Early lactation cows	Mid lactation cows	P
Glucose (mmol/l)	2.29±0.48	2.76±0.43	< 0.05
BHB (mmol/l)	1.59±0.25	0.91±0.16	< 0.05
NEFA (mmol/l)	0.38±0.29	0.13±0.04	< 0.05
TG (mmol/l)	0.12±0.02	0.15±0.04	NS
AST (U/l)	69.46±27.54	39.31±18.90	< 0.05

Significant changes in most blood metabolites between the experimental groups of cows were found. Blood glucose concentration was significantly lower ($P<0.05$) in early lactation cows as compared to mid lactation cows. Biochemical testing for lipids and ketone bodies in the blood serum showed significantly higher values ($P<0.05$) of NEFA and BHB in early lactation cows as compared to those in the blood sera of mid lactation cows. In addition, AST activities were markedly increased in puerperal cows compared to mid lactation females ($P<0.05$). Mean values for blood TG in early lactation cows were lower, but no significant differences ($P>0.05$) between the two groups.

The correlation coefficients among the biochemical indicators calculated for all cows in this experiment are summarized in Table 3

Table 3. Correlation coefficients for the biochemical indicators calculated for all cows in the present study. Significant correlations (p<0.05) are marked with asterix (*).

	NEFA	BHB	TG	AST
Glucose	r= -0.35*	r=-0.47*	r=0.65*	r=-0.23
NEFA		r=0.39*	r=-0.21	r= 0.34*
BHB			r=-0.36*	r=0.15
TG				r=-0.04

Significantly negative correlations (P<0.05) were observed between BHB and glucose, BHB and TG, NEFA and glucose. Significantly positive correlations (P<0.05) were observed between NEFA and BHB, NEFA and AST, glucose and TG.

Intensive peripartal lipid mobilization and ketogenesis are sufficient for a series of compensatory metabolic processes with changes in blood metabolic profile during early lactation in healthy cows *(Cincović et al., 2012)*. In early lactation cows, NEFA and BHB values were significantly higher (P<0.05) than in mid lactation cows. NEFA concentrations > 0.40 mmol/l indicate problems with energy balance and subsequent intensive lipomobilization *(Oetzel, 2004)*. According to this report, 6 (40%) out of 15 early lactating cows and none of mid lactation cows showed evidence of high lipomobilization (NEFA >0.40 mmol/l) in the present study. Given the fact that serum NEFA concentrations > 0.70 mmol/l are associated with ketosis *(Oetzel, 2004),* 2 (13.3%) early lactating cows and none of mid lactation cows in the present study had NEFA concentrations above the value indicative of subclinical ketosis. Subclinical ketosis also may be diagnosed when serum BHB concentrations are above 1.2 mmol/l, while clinical ketosis is associated with BHB concentrations above 2.6 mmol/l *(Duffield, 2000; Oetzel, 2004)*. In the present study, 14 (94.4%) early lactation cows and none of mid lactation cows had serum BHB concentrations above 1.2 mmol/l. These data suggest that serum NEFA (13.3%) could be less efficient indicators of subclinical ketosis than serum BHB (94.4%) in dairy cows during early lactation. The data presented here show that serum NEFA may be used for detecting high lipomobilization, but not subclinical ketosis. This is in agreement with *(Duffield, 2000),* who stated that the use of NEFA is a better indicator of energy imbalance in prepartum animals than BHB, but BHB is more useful postpartum. In the present study, a significant positive correlation (r=0.39, P<0.05) was established between NEFA and BHB in the sera, suggesting that both parameters are helpful indicators of energy balance during lactation. Additionally, the relationship between BHB and NEFA may be inferred from the significant correlations between BHB and glucose (r=-0.47; P<0.05), and BHB and TG values (r=-0.36; P<0.05). Blood glucose values in mid lactation cows were within the physiological range 2.5 - 4.2 mmol/l *(Radostis et al., 2000),* whereas hypoglycemia was detected in early lactation cows. In the present work, 10 (66.6%) early lactation cows and 5 (33.3%) mid lactation cows had blood glucose concentrations below 2.5 mmol/l.

Fat infiltration into the liver may also affect the concentration of some blood components. Serum level of TG, is indicator of hepatic functionality, and decreases in their concentration may suggest fat infiltration in the liver *(Herdt et al., 1983; Sevinc et al., 2003; Đoković et al., 2007, 2011)*. The concentration of serum TG was significant lower (P<0.01) in ketotic cows compared to healthy cows *(*

Đoković et al., 2007). These results are showed that TG accumulate in the liver cells of ketotic cows and cause their blood values to decrease. TG concentrations below 0.12 mmol/l were found in 7 (44%) early lactation cows and 2 (13.3%) mid lactation cows. In the present study, TG in the blood was low (0.12 ± 0.02 mmol/l vs 0.15 ± 0.04 mmol/l) in both groups of cows, but without significant difference ($P>0.05$). This study was showed a posibility of the development a fat infiltration of the liver in early lactation cows and was confirmed a significant correlation between TG and glucose ($r=0.65$; $P<0.05$). However, all cows suffered from subclinical ketosis (BHB > 0.12mmol/l) according to the criterion cited above *(Sevinc et al., 1998; Oetzel, 2004; Gonzales et al., 2011)* and 6 or 40% of early lactation cows (NEFA > 0.40 mmol/l), were found to have TG values less than 0.12 mmol/l and glycemia below 2.5 mmol/l..

When fat infiltrates the liver, a lesion appears in hepatic tissues, and the levels of enzymes that indicate liver injury (AST, GGT, and LDH) are generally augmented *(Pechova et al., 1997**;** Lubojacka et al., 2005).* AST values in the present study were statistically higher ($P<0.05$) in early lactation cows than in mid lactation cows. If AST activity higher than 100 U/l is indicative of hepatic lesions *(González et al., 2011).* These are result that 2 (13.3%) early lactation cows in our study suffered from some degree of hepatic lesions, probably due to fat infiltration. Also, a positive correlation was observed between AST activity and NEFA values. In the present study, all data concerning serum AST activities suggested that the process of lipomobilization was sufficient to cause liver lesions in the early lactating cows. Possible changes in the liver function may have deleterious effects on the metabolism of these animals, and may impact milk production or reproduction.

Conclusion

In conclusion**,** on the basis of changes of blood biochemical metabolites, this study suggests that early lactation cows had metabolic disturbances, which were associated with ketosis, and some degree of hepatic lesions, probably due to fat infiltration. They can serve as useful indicators of the metabolic status of dairy cows during lactation.

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Procenjivanje metaboličkog statusa kod mlečnih krava na početku i sredinom laktacije na osnovu promena biohemijskih indikatora krvi

Z. Ilić, R. Đoković, V. Kurčubić, M.P. Petrović, V. Caro-Petrović, J. Stojković, B. Ristanović

Rezime

Cilj ovog rada je bio da se proceni metabolički status kod mlečnih krava Simentalske rase na početku laktacije (n=15) i sredinom laktacije (n=15) na osnovu promena biohemijskih indikatora krvi. Uzorci krvi su uzeti od svih ispitivanih krava da bi se odredile koncentracije beta-hidroksi-buterne kiseline (BHB), neesterifikovanih masnih kiselina (NEFA), triglicerida, glukoze i aktivnost aspartat-transaminaze. Krave na početku laktacije su imale značajno veće vrednosti beta-hidroksi buterne kiseline ($P<0.05$) i neesterfikovanih masnih kiselina ($P<0.05$) u krvi, nižu glikemiju ($P<0.05$) i niže vrednosti triglicerida ($P<0.05$) u odnosu na vrednosti kod krava na sredini laktacije. Visoka lipomobilizacija (NEFA >0.4 mmol/l) i subklinička ketoza (BHB >1.2 mmol/l) je utvrđena kod 6 (40%), odnosno 14 (94.4%) krava na početku laktacije i kod nijedne krave na sredini laktacije. Aktivnost aspartat-transaminaze iznad 100 IJ/l je utvrđena kod 2 krave na početku laktacije i kod nijedne krave na sredini laktacije. Koncentracije triglicerida u krvi ispod 0.12 mmol/l utvrđene su kod 7 (44%) krava na početku laktacije i kod 2 (13.3%) krave na sredini laktacije Koncentracije glukoze ispod 2.5 mmol/l su utvrđene kod 10 (66.6%) na početku laktacije i kod 5 (33.3%) krava na sredini laktacije. Vrednosti glukoze, triglicerida, beta-hidroksi buterne kiseline, neesterifikovanih masnih kiselina u krvi i aktivnost aspartat-transaminaze, pokazuju da krave na početku laktacije imaju metaboličke poremećaje, koje su povezane sa ketozom i nekim stepenom oštećenja ćelija jetre, verovatno kao posledica masne infiltracije. Rezultati ukazuju da ispitivani biohemijski indikatori krvi mogu biti korisni u procenjivanju metaboličkog statusa kod mlečnih krava tokom laktacije.

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WELFARE INDICATORS OF DAIRY COWS - SELECTION AND IMPLEMENTATION IN ASSESSMENT

D. Ostojić-Andrić[1], S. Hristov[2], M.M. Petrović[1], V. Pantelić[1], S. Aleksić[1], D. Nikšić[1], M. Dokmanović [3]

[1]Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080, Belgrade-Zemun, Republic of Serbia
[2] Faculty of Agriculture, Nemanjina 6, 11080, Belgrade-Zemun, Republic of Serbia
[3] Faculty of Veterinary Medicine, University of Belgrade, Bulevar Oslobođenja 18, Belgrade, Republic of Serbia
Corresponding author: andricdusica.iah@gmail.com
Invited paper

Abstract: Definition, implementation, analysis of relevance and development of standards of animal welfare on cattle farms have become very important issues in the late 20th and early 21 century. Today, the most widely accepted definition of welfare is that it is the condition of an animal as a response to its attempts to cope with the effects of the environment. Depending on the type, duration and intensity of action of stimuli and the condition of the animal this struggle can be successfully or unsuccessfully completed in which case the animal welfare is endangered. But how do you assess the welfare as satisfactory and when not? In declaration of welfare as a scientific field, the turning point was the observation of its measurable character or the fact that as a result of the reaction to the effects of the stimulus in the organism of the animal changes occur at the physical, physiological, behavioural and emotional level that can be measured. Traits mentioned belong to output or animal-based welfare indicators which have a primary role in the modern methods of assessment. Unlike them, input indicators relate to information resources (resource - based) and applied management (management - based) and are important as additional information in the evaluation. Problems and the importance of selecting indicators in the assessment of the quality of welfare are still present but the practical implementation of a wide variety of assessment methodologies over time should enable better perception, analysis and even synthesis of the most relevant indicators and targets for evaluation of the different methods. This paper presents an overview of the selection, implementation and use of indicators to assess the welfare of dairy cows with a special emphasis on two current methodologies.

Key words: welfare, quality, dairy cows, indicators, assessment, methods

Introduction

Today, taking into consideration the gravity and exposure to influences that threaten the welfare and the number of farmed animals, problem of welfare of dairy cows is one of the most pronounced in Europe (*Hristov et al., 2011a; Ostojić - Andrić et al., 2012; Nakov et al ., 2012).* Protecting the welfare of dairy cows is an extremely complex issue that involves different aspects and requires fundamental changes in the breeding programs and management systems (*Hristov et al., 2011b; Ostojić - Andrić et al., 2011, Karasek et al., 2012*) The issue of animal welfare mainly occupies those who are directly or indirectly involved in the production of food - consumers and producers of animal products. In addition, the protection of the welfare of dairy cows is associated with environmental issues, sustainable development, and a range of medical, hygienic, economic and social problems of the society.

The definition and the concept of welfare

The concept of animal welfare involves both ethical and scientific dimension and therefore requires an unambiguous definition (*Duncan, 2003*). Today, the most widely accepted definition of welfare is that it is a condition of an animal as a response to its attempts to cope with the effects of the environment (*Broom, 1986*). In addition, to cope means to fight to gain control of mental and physical stability. There are different coping strategies from behavioural, physiological, immunological and other that are managed by the brain. Feelings, such as pain, fear, and various forms of pleasure, also can be a part of coping strategy. In modern conditions of production, the animals are under the influence of a lot of stimuli to which they have to adjust in order to maintain their physical, physiological and emotional integrity. Therefore, for the maintenance of animal welfare key issue is how to fulfil their needs, i.e. freedoms: (1) Freedom from hunger, thirst and malnutrition; (2) Freedom from thermal and physical distress; (3) Freedom from pain, injury and diseases; (4) Freedom to express natural behaviours; and (4) Freedom from unpleasant emotional experiences (*FAWC, 1979*). Figure 1 shows the general concept of animal welfare, which includes adaptation of physiology and behaviour in order to maintain proper health condition which, as the final result, has increased productivity (*Sejian et al., 2011*).
Sejian et al. (2011) Blokhuis et al. (2003)

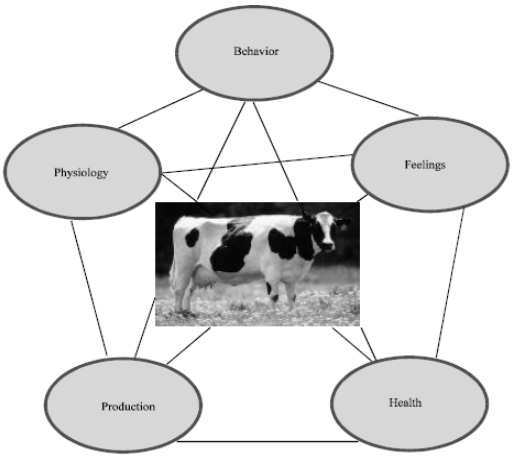

Figure 1. General concept of animal welfare

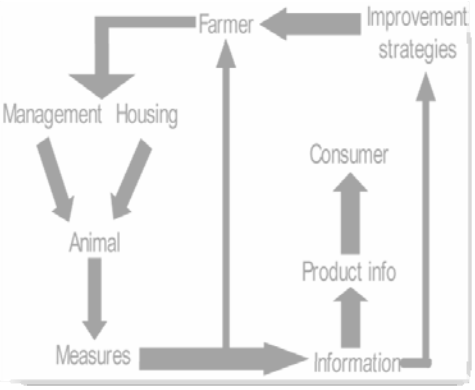

Figure 2. Strategy of improvement of animal welfare on farms

Goals of the animal welfare quality assessment

According to *Vučinić (2006)* goals for evaluation of the welfare of domestic animals are following: a) to advise owners and breeders to improve animal welfare, b) to compare the conditions of farming and exploitation of animals to the conditions stipulated in the legislation, c) based on the assessment of animal welfare to apply appropriate certification scheme in the production of food of animal origin (for example, "organic food", "dairy products from pasture/grazing farming system of dairy cows", etc..) and d) based on the evaluation welfare to adapt and improve legislation governing animal welfare.

Methods of animal welfare assessment

The overall assessment of animal welfare according to *Botreau et al. (2007a, 2007b)* is a complex problem that requires evaluation of a constructive strategy for the integration of information obtained by various measurements of animal properties and environmental parameters. The method of animal welfare assessment at the same time depends on the concept of welfare, applied indicators and the way to identify and analyze the data. For the definition and use of indicators in practical terms are especially important works of *Bartussek (2000), Bracke et al. (2001), Bracke and Hopster (2006)* and *Meagher (2009)*. Today there is a great knowledge of the indicators of welfare, but to define the methods for evaluation of welfare, the key issue is to reduce their number and include only the most important and reliable indicators. The method of welfare assessment should provide ease and convenience of reference (not expensive, does not take long, does

not disturb the normal process of technology on the farm), while allowing an objective, valid and repeatable results (*Hörning, 2001; Spoolder et al., 2003)*.

Methods for evaluating of animal welfare can be roughly divided into so-called input methods based on information about environmental conditions and resource-output methods that use information about the animals themselves (*Main et al., 2003*). Welfare assessment, which relies on the assessment of resources such as the type of bedding, food, hygiene and so on, is often simple and fast but it represents only the prerequisite of welfare and not the absolute guarantee. That is why today more importance is given to the methods based on information about animals that include determining of the physical condition of animals (lameness, injuries to the skin), observation of behaviour (fear, liveliness, aggression) and data collection (e.g. on morbidity, mortality, productivity). Output methods are more demanding in terms of data collection and processing as well as the time required but their application still provides reliable results on the quality of welfare (*Johnsen et al., 2001*). These authors concluded that the best method for welfare evaluation should incorporate both of these types of indicators (input and output).

To date, numerous methods have been developed to assess the welfare of farm animals some of which are included in the legislation of a country or region, and actively implemented, supplemented and questioned. Such is the case with following methods: Animal Needs Index – ANI, EFSA method of risk assessment for animal welfare, Protocol for assessing the quality of welfare (*Welfare Quality Assessment Protocol for Cattle, 2009*). In our country, a method has been developed for assessing of the welfare of dairy cows in the project TR 20110.

EFSA method of risk assessment to dairy cows' welfare

This method allows the assessment of risks to animal welfare, taking into account the different systems of breeding, management, species and categories of animals as well as various aspects of welfare in the specific scenario of exposure to a factor. At the beginning, the keyword in the methodology - hazard, indicated each adverse effect, which increased the risk to welfare, was replaced by a new term - a factor that is related to any aspect of the environment and the changes that can have a positive or negative impact on the welfare state. Factors that influence the state of welfare include sources in the environment available to animals (space, equipment, places where they can lie down) as well as farm management and can be determined by the assessment of appropriate inputs (*resource - based and management - based*) indicators. Animals, depending on their characteristics (breed, gender, age), provide an answer on the effects of these inputs that can be determined through the output (animal - based) indicators. As previously stated, purpose of the assessment of indicators is reflected among other things in defining recommendations for the improvement of welfare. The specificity of this method

lies in the fact that based on the estimated risks specific recommendations for the improvement of welfare are given. The *EFSA report (2012)* stated the 105 recommendations relating to various segments of securing the welfare of dairy cows based on an estimate of the risk of the most common hazards to the welfare of caused by the way of breeding, feeding, management and genetic selection effects on incidence of mastitis, metabolic, reproductive, locomotion , behavioural and emotional disorders.

The Welfare Quality ® Assessment Protocol for Cattle (dairy cows)

The *Welfare Quality ® Assessment Protocol for Cattle (2009*) is a scientific method for assessing the welfare of farm animals that was obtained from the sixth framework program of the European Union (Sixth Framework Programme - FP6) entitled The Welfare Quality ® Project. The main objective of the project was to develop a standardized methodology for assessing the welfare, practical strategies and measures for its improvement, and standardized methodology that would enable for assessed welfare to simply be translated into easily understandable information about the product (Figure 2). The protocol for assessing the quality of welfare initially was based on the definition of welfare by *Duncan and Petherick (1991)* according to which emotions and subjective feelings of animals are of primary importance, and alternative concepts such as natural behaviour and/or environment of less importance. Therefore, the protocol for the assessment of the quality of welfare includes a number of indicators of welfare, which are primarily based on information about the animals and to a lesser extent on the resources or farm management.

Choice of indicators, their relevance, feasibility and reproducibility were considered as the highest priority (*Veissiere et al., 2007*). Eventually, this method is defined by the assessment of 4 basic principles, 12 key criteria and over 30 indicators of welfare and has been developed for cattle (dairy cows, beef cattle and calves), pigs (sows and fatteners) and poultry (laying hens and broilers). Principles and assessment criteria are the same for all types of farm animals, while the indicators of welfare are species specific. The final assessment of the welfare state on the farm is obtained by scoring (giving points) of indicators whose sum specifies one of four qualitative categories of welfare under the respective criteria and principles. The total score classifies the welfare into following categories: unsatisfactory, acceptable, appropriate/adequate and excellent. Starting from a multidimensional concept of welfare, the project highlights the importance of the criteria, i.e. dimensions of welfare in which the application of specific mathematical operations (*Choquet integral*) provides that certain criteria are given more importance relative to the other, while at the same time the possibility of compensation between them is minimized.

Indicators of welfare quality

Depending on the intensity and duration of the stressors and the ability of animals to respond appropriately to environmental impacts, coping mechanisms may operate successfully when the fight is completed or unsuccessfully - when the animal is threatened. However, how to determine whether the welfare of an animal is satisfactory or endangered? For scientists in the field of welfare it is extremely important that the quality of welfare can be measured. Measurability of the quality of welfare stems from the fact that as a result of reactions to the various challenges of the environment, animals exhibit the above-mentioned coping strategies that can be used as indicators of their welfare. Today a wide range of indicators used to assess the quality of the welfare of dairy cows are known, which can be generally classified into two major groups - input and output indicators. Input indicators include all indicators indicating to housing and management conditions, and include resource - based and management - based indicators that are relatively easy to measure. On the other hand, output or animal - based indicators are the result of attempts of animals to cope with their environment and result in certain physical, physiological and mental changes that are also measurable. Table 1 provides an overview of these groups of indicators by *Welfare Quality Assessment Protocol for Cattle (2009).*

Table 1. Types of indicators used for assessment of welfare of dairy cows

nimal - based	body condition, time needed to lie down, collision with housing equipment, lying partly or completely outside the lying area, cleanliness of udders, flank, upper and lower legs, lameness, integument alterations, coughing, nasal discharge, ocular discharge, hampered respiration, diarrhea, vulvar discharge, milk somatic cell count, dystocia, downer cows, mortality, agonistic behaviour, avoidance distance, emotional state
esource -	water provision, cleanliness of water points, water flow, functioning of water points, thermal comfort, presence of tethering, access to outdoor loafing area or pasture
anagement -	disbudding /dehorning, tail docking

Welfare Quality® Assessment Protocol for Cattle (2009)

Animal - based indicators

Animal - based indicators are response of animal to farming conditions. Therefore, their evaluation can determine the current state of animal welfare, including the impact of management and environment. These indicators can be

determined directly or indirectly from animals and based on data available on the farm. Animal - based indicators are intended for:

- assessing the level of disruption caused by injury, disease and malnutrition.
- providing information about the needs of animals and affective states such as hunger, pain and fear often measured by the strength of motivation and aversion in animals
- assessment of the degree of physiological, behavioural, immunological and other changes that animals exhibit in response to a variety of challenges from the environment.

Assessment of the animal - based indicators presented in Table 1 provides information on health and nutrition, hygiene, comfort, health and mortality, as well as the emotional state and the presence of behaviour (*Welfare Quality® Assessment Protocol for Cattle, 2009)*.

Resource - based indicators

Resource - based indicators include all those indicators relative to the conditions of the farm in terms of farming space (structure, size, floors, bedding, equipment, hygiene), access to outlets or pasture, water supply, ventilation, thermal regime and so on. The importance of this group of indicators is reflected in the following:

- better understanding of the importance of animal - based indicators to assess the welfare;
- as a replacement for one animal - based indicators that are not reliable or suitable for the assessment on the farms;
- as risk factors to animal welfare.

In a study by *Algers et al. (2009)* initially over 90 potential resource - based indicators have been tested, and ranked according to their importance for the welfare and convenience of application in monitoring the welfare on farms. The authors state the most important indicators in terms of dairy cows welfare:

1. Indicators of satisfactory water supply of cows (water supply, water flow, purity and safety of drinkers)
2. Thermal comfort of cows
3. Freedom to move (the presence of tie system and the possibility for use of free range or pasture and demonstrating other forms of behaviour in cows in the pasture).

The size or capacity of the farm according to some authors *(Rauw et al., 1998, Royal et al., 2000*) is also an important factor to be taken into consideration when assessing the resource - based quality indicators for welfare on dairy farms.

Management - based welfare indicators

These indicators relate to the actions and measures that are implemented in the management of the farm. Their biggest importance in the assessment of welfare on farms is reflected in the supplementation of data provided by the animal - based indicators. In addition, they allow the breeders or farm managers to be provided feedback on risk of applied management to the welfare of cows with the ability to apply appropriate corrections (*Waiblinger et al., 2009*). As potential management - based indicators numerous indicators can be stated such as the characteristics of the applied systems of housing, hoof treatment procedure, the presence of equipment-brushes for hygiene, mastitis control, the method of recording data on farm, shortening of the tail, dehorning and the use of anaesthesia and analgesia. *Waiblinger et al. (2009)* reported a list of 53 potential indicators whose assessment may be based on three methods: interview of managers or breeders, direct observation of animals and management systems, inspection of records containing information on the farm or a combination of these methods. After the study of the significance and reliability of the indicators and the evaluation of simplicity of their performance on a farm, finally only two were included in the *Welfare Quality® Assessment Protocol for Cattle (2009):* dehorning and shortening the tail.

Selection of indicators in welfare assessment

When defining a methodology for the assessment of welfare, we encounter a wide range of indicators that could be used. *The EFSA report (2012)* disclosed over 70 animal - based indicators, stating that it would be highly unrealistic and unnecessary to use all the indicators at each assessment. It is recommended that in such a set of indicators only those necessary to evaluate specific segments of the welfare are selected, e.g. health condition and nutrition, depending on the purpose of assessment (compliance with legal regulations, improvement strategies, etc.). On the other hand, in establishing *the Welfare Quality® Assessment Protocol for Cattle (2009)*, after a thorough examination and assessment of validity, reliability, feasibility and ease of assessment of indicators in farm conditions, only 31 indicators of relevant importance for the assessment of the quality of welfare were selected. The results of these researches are presented within the *Welfare Quality Reports No.11. (2009)*. The exception is the criterion - the thermal comfort of cows, which is a measure of the principles of good posture assessment, it is still considered and reviewed.

Significance and related indicators in the assessment of welfare

In addition to the selection of indicators that will in the best way describe the state of the welfare of dairy cows, it is a question of their individual importance or weight that each has for the overall welfare assessment. *Webster (2005)* states 'ranking' of top 10 indicators of welfare while in *the EFSA report (2012)* the five

most important indicators are stated (Table 2). In *the Welfare Quality® Assessment Protocol for Cattle (2009*), this problem was solved by applying the so-called weight coefficients (WC) associated with the indicators of welfare (Table 3). Thus, for example, in assessing the proper nutrition as one of the principles of welfare, absence of long-term thirst is allocated greater importance in relation to the absence of hunger. It is important to point out that the importance of indicators is defined on the basis of their impact on the emergence of various welfare problem.

Table 2. Comparative rank list of indicators according to different source

Rank	**I n d i c a t o r s**	
Source	*Webster(2005)*	*EFSA(2012)*
1	Observing lameness	Observing lameness
2	Examining health records	Observing hock, knee skin lesions and swelling
3	Observing disease	Colliding with equipment
4	Observing mastitis	Teat injuries
5	Observing general demeanour	Observing mastitis
6	Scoring body condition	
7	Observing stockmanship	
8	Observing lying behaviour	
9	Examining production records	
10	Observing skin lesions	

Table 3. Relationships in regard to the significance of indicators within the various segments of welfare

	P r o v i s i o n o f																				
	Good feeding			Good hosing					Good health					Appropriate behaviour							
	Absence of thirst	>	Absence of hunger	Ease of movement	>	Comfort around resting	>	Thermal comfort	Absence of disease	>	Absence of pain induced by management procedures	>	Absence of injuries	Positive emotional state	>	Good human - animal relationship	>	Expression of social behaviours	>	Expression of other behaviours
WC	**0.26**	>	**0.09**	**0.23**	>	**0.20**	>	**0.18**	**0.19**	>	**0.10**	>	**0.06**	**0.16**	>	**0.11**	>	**0.10**	>	**0.09**

Welfare Quality® Assessment Protocol for Cattle (2009)

Conclusion

Assessment of welfare quality of dairy cows should allow precise identification of risk factors for welfare that lead to undesirable values of welfare indicators, in order to define recommendations for their elimination or at least reduction. To date, however, ideal methodology for evaluation of welfare of dairy cows has not been defined, although in the method of Welfare Quality Protocol and EFFS Risk Assessment great efforts were achieved. Within these methods, of numerous welfare indicators, the most important ones were identified in respect to the assessment of individual segments or providing overall welfare assessment. Also, the score by Welfare Quality protocol takes into account the growing importance of certain indicators in relation to others and minimizes the compensation between them. The ease of this method of assessment is certainly of great practical importance, as well as the specific results of the evaluation in the form of categorization of rated farms. The advantage of a method that is based on the assessment of risk to welfare is reflected in the fact that indicators viewed in the assessment are directly associated with appropriate recommendations, which enables faster action. It can be concluded that the formulation of modern, science-based, method of evaluation of the quality of welfare emphasis is placed on the animal - based indicators, assessing their relevance and ease of use in the assessment. Given the complexity and duration of the overall welfare assessment on farms, the idea of partial welfare assessment based on the purpose of assessment is more often mentioned, as well as the ability, based on the evaluation of provision of certain welfare segments, its overall value is assessed. In spite of the fact that the problems and the importance of selecting indicators in the assessment are still present, broad practical implementation of these assessment methodologies over time should enable better perception, analysis and even the synthesis of the most relevant indicators and assessment targets from different methods.

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Indikatori dobrobiti mlečnih krava - odabir i primena u oceni

D. Ostojić-Andrić, S. Hristov, M.M. Petrović, V. Pantelić, S. Aleksić, D. Nikšić , M. Dokmanović

Rezime

Definisanje, uvođenje, analiza relevantnosti i razvoj standarda zaštite dobrobiti životinja na farmama goveda postale su veoma značajne teme krajem 20. i početkom 21. veka. Danas najšire prihvaćena definicija dobrobiti je da ona predstavlja stanje životinje nastalo kao odgovor na njene pokušaje da se izbori sa uticajima iz životne sredine. Zavisno od vrste, trajanja i intenziteta delovanja stimulusa kao i od stanja same životinje ova borba može biti uspešno ili neuspešno okončana u kom slučaju je dobrobit životinje ugrožena. Ali kako proceniti kada je dobrobit zadovoljavajuća a kada ne? Za deklarisanje dobrobiti kao naučne oblasti prelomni trenutak bila je konstatacija njenog merljivog karaktera odnosno činjenica da se kao rezultat reakcije na dejstvo stimulusa u organizmu životinje odigravaju promene na telesnom, fiziološkom, bihejvioralnom i emocionalnom nivou koje se mogu izmeriti. Pomenute osobine pripadaju output ili animal-based indikatorima dobrobiti koji u savremenim metodama ocene imaju primarnu ulogu. Za razliku od njih, input indikatori odnose se na informacije o resursima (recourse - based) i primenjenom menadžmentu (management - based) i od značaja su kao dopunska informacija u oceni. Problemi odabira i važnosti indikatora u oceni kvaliteta dobrobiti još uvek su prisutni ali bi široka praktična implementacija različitih metodologija ocene tokom vremena trebala da omogući bolje sagledavanje, analizu pa i sintezu najrelevantnijih indikatora i ciljeva ocene dobrobiti iz različitih metoda. U radu je iznet pregled problematike odabira, primene i korišćenja indikatora u oceni dobrobiti mlečnih krava sa posebnim osvrtom na dve aktuelne metodologije.

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THE DAIRY FARM RISK ASSESSMENT AND DEVELOPMENT OF BIOSECURITY PLAN

S. Hristov[1], B. Stanković[1], N. Maksimović[2], D. Ostojić-Andrić[2], Z. Zlatanović[3]

[1]University of Belgrade - Faculty of Agriculture, Nemanjina 6, 11080, Belgrade-Zemun, Republic of Serbia
[2]Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080, Belgrade-Zemun, Republic of Serbia
[3] Higher Agricultural School, Prokuplje, Republic of Serbia
Corresponding author: hristovs@agrif.bg.ac.rs
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Abstract: This paper describes the most important aspects regarding risk assessment and development of biosecurity plan in dairy farm production. Risk assessment is the way of determining the presence, distribution, and severity of a given disease. After risk areas have been identified, appropriate control measures can be enacted. The risks of introducing infectious and parasitic diseases can be reduced by carefully assessing the disease status of the farm, inspecting animals prior to introduction, segregating and observing introduced animals before allowing contact with other animals, purchasing only semen and embryos from reputable sources, checking possession of veterinary health certificates, ensuring that animal movements are notified and limiting contact with wildlife, vermin and feral animals. Any biosecurity plan should provide all aspects regarding an appropriate farm location, breeding only one species in the production area, minimizing risks to the cattle health, primarily from the environment and the production unit, additional biosecurity measures in the case of emergency of the disease, the hygienic-sanitary measures in all facilities and closest environment, imminent danger of an disease outbreak, keeping records on the animals introduction into production area, morbidity, treatments, mortality, relocation and sales of the animals.

Key words: dairy farm, risk assessment, biosecurity plan

Introduction

Nowadays, as dairy cattle production has intensified, the risk of developing production-limiting diseases, such as contagious mastitis *(Dinsmore, 2002)*,

Johne's disease *(Benjamin et al., 2010)*, enzootic bovine leucosis (*Gramig et al., 2010*) and bovine viral diarrhoea *(Smith and Grotelueschen, 2004)*, has increased. Also, more intensive dairy cattle production practices increase *E. coli (Barrington et al., 2002)* and *Salmonella* risk *(Davison et al., 2006)*, as well as other causing agents *(Hennessy, 2007; Stanković et al., 2012)*. In fact, any contagious disease can potentially reduce dairy cattle productivity and the animal welfare, increase veterinary and labour costs, impact product quality and consumer confidence, and reduce the dairy herd and farm's value *(Maunsell and Donovan, 2008; Sibley, 2010; Mee et al., 2012; Toma et al., 2013)*. Dairy farmers are continually identifying and managing risks in order to run good business that produce quality products while caring for their people, animals, land and the wider environment *(Wells, 2000)*. Substantially, dairy cattle farm biosecurity refers to all management practices that reduce the probability of infectious and parasitic diseases which will be carried onto the farm by animals or people and also the spread of infectious and parasitic diseases on farms *(Dargatz et al., 2002; Ramirez-Villaescusa et al., 2010; Sibley, 2010)*. Biosecurity is particularly important against the threat of so-called ″management diseases″, such as calf pneumonia and calf scours which cost the dairy productions large sums of money every year through death, poor growth, poor feed conversion and the cost of medication *(Uttenthal et al., 1996; Barrington et al., 2002)*.

The responsibility for dairy farm-level biosecurity belongs to the producer or herd owner *(Hoe and Ruegg, 2006; Gunn et al., 2008; Kristensen and Jakobsen, 2011; Toma et al., 2013)*. A successful biosecurity plan must address isolation of new animals brought to the farm, isolation of sick animals, management of the movement of people, animals, and equipment, and procedures for cleaning and disinfecting facilities *(Morley, 2002; Moore et al., 2008; Uhlenhoop, 2007, Stanković et al., 2010)*.

The aim of this paper was more detailed discussion risk assessment and development of biosecurity plan in dairy farm production on the basis of current literature data and experience of the authors.

Biosecurity on dairy farms – general aspect

The concepts of biosecurity on dairy farms are not new *(Anderson, 1998)*. An introduction to biosecurity of dairy cattle operations can be found in the paper by *Dargatz et al. (2002)*. In our country, the standards for biosecurity in the cattle production in the form of guidance were made *(Anon., 2011)*. The guidance on cattle biosecurity includes standards developed over the last two years, as a series of management practices to help farmers minimize and control disease risk entering their farms, spreading within the farm or to neighbouring farms. In

addition, some important aspects of standards for our country were described in the papers by *Hristov et al. (2009)* and *Hristov and Stanković (2009a)*. These standards provide a framework for on-farm biosecurity that can be applied to all dairy farms, regardless of size or geographical location. Implementation of biosecurity measures in Serbian farm production was described in details *(Reliić et al., 2006; Hristov et al., 2007; Plavsić et al., 2011)* as well as welfare and biosecurity indicators evaluation in dairy production *(Hristov and Stanković (2009b)*.

In the last few years implementation of biosecurity measures on dairy farms was discussed in developed countries, for example in Ireland *(Sayers et al., 2012)* and Canada *(Anon., 2013)*. *Kristensen and Jakobsen (2011)* described Danish dairy farmers' perception of biosecurity. Opinions and practices of Wisconsin dairy producers about biosecurity and animal well-being were given in the paper by *Hoe and Ruegg (2006)*. The most important biosecurity measures and failures in cattle farms were described by *Hristov et al. (2002)* and *Stanković and Hristov (2009)*, respectively. A behavioural economics analysis as determinants of biosecurity behaviour of British cattle and sheep farmers were given in paper by *Toma et al., (2013)*. Biosecurity hazards and risks on dairy operations were considered in the paper by *Wells (2000)* and *Mee et al. (2012)*. As very significant it was emphasised that the evaluation process should be done in consultation with herd veterinarian, extension specialist and nutritionist, and consider facility design and layout, type, size of operation, farm team, herd disease status, and animal health and facility management strategies *(Gunn et al., 2008)*.

Risk assessment

Risk assessment is a way of determining the presence, distribution, and severity of a given disease. After risk areas have been identified, appropriate control measures can be enacted *(Wells, 2000; Morley, 2002; Hristov and Stanković, 2009b; Mee et al., 2012)*. Acceptable levels of risk for a dairy farm will be determined by what products are sold or what may be sold from the farm in the future. Understanding which diseases are important for the sale of each of the products and how disease may enter and spread within the animal groups is the next step. This is followed by close evaluation of methods to prevent the disease from entering the dairy herd from sources outside the farm. Also, the risk can be categorized by source of possible infection (other livestock, visitors, or wildlife), area of the farm (maternity pens, facilities for newborns and young stock, and feed storage areas), or by susceptibility of animals (calves, young stock, and periparturient cows). If key areas can be identified, they are often marked as critical control points. Actions taken at these critical control points are the most

effective way of implementing a biosecurity plan in a dairy farm *(Villarroel et al., 2007)*.

It is well known fact that diseases can be introduced in purchased animals and those that have been off the farm for a while, for example on pasture, sale yards and at shows *(Morley, 2002; Sibley, 2010)*. Biosecurity and risk management for dairy cattle replacements were given by *Maunsell and Donovan (2008*). The risks of introducing disease in mentioned ways can be reduced by carefully assessing the disease status of the originating farm, inspecting animals prior to introduction, segregating and observing introduced animals before allowing contact with other animals, only purchasing semen and embryos from reputable sources, checking possession of veterinary health certificates, ensuring that animal movements are notified and limiting the animals contact with wildlife, vermin and feral animals (*Villarroel et al., 2007; Sayers et al., 2012)*.

Another important biosecurity issue is minimizing the occurrence and spread of animal diseases through preventive herd health program and frequent observation of the animals to ensure early detection of diseases and prompt treatment. Key elements of a herd health program include vaccinations, comprehensive mastitis prevention program, work with neighbours to minimise spread of diseases, reporting unusual outbreaks of disease, and plan for prompt disposal of dead stock *(Hennessy, 2007; Maunsel and Donovan, 2008; Gramig et al., 2010; Sibley, 2010)*.

A significant threat to dairy farm biosecurity can be brought in by products such as stock feed, bedding materials, water, road and track materials and fertilizers that have the potential to bring in disease agents and weed seeds. These risks can be minimised by purchasing stock feeds from reputable suppliers, ensuring that stock feeds are animal origin products free, limiting access to water sources, observing withholding times for introduced products (such as poultry shed effluent and animal manure fertilizers) and checking sources of farm inputs prior to purchase *(Burton, 2009; Hristov and Stanković, 2009a; Sibley, 2010; Anon., 2011; Anon., 2013)*.

From the very beginning of farm biosecurity considerations is believed that people and vehicles *(Kirk et al., 2003; Brennan and Christley, 2012)* both have the potential to introduce weed seeds and infectious disease agents. It is emphasized that different categories of visitors, contractors and service personnel pose different levels of risk and farmers need to have strategies to manage those that pose the highest risks. The strategies to manage the risks in dairy farm might include limiting unnecessary movements over the farm, established restricted areas of access for farm contractors and transport operators, providing facilities for farm contractors and visitors to clean boots and equipment, taking care to minimise risks of owner spreading disease agents when visiting other properties, sale yards or

other places that have animals of unknown disease status *(Uhlenhoop, 2007; Stanković et al., 2011)*.

It is not disputable that effluent and waste materials have the potential to spread disease on the farm or to neighbours. These risks can be reduced by an effluent use plan that includes controls to prevent the potential spread of disease, a disposal plan for potentially contaminated waste materials such as bedding, used syringes and dumped milk, capturing, containing and disposing of effluent from the dairy and yards within the boundaries of the farm and fencing effluent storage areas to prevent access by people and livestock *(Burton, 2009)*.

Many times it has been proven that neighbours can be a source of animal diseases (*Hennessy, 2007*). Every farm has different relationships with neighbours and farmers need to assess risks posed by animal movement through fences, contact over fences and from disease agents and weed seeds washing across fence lines. The risks can be reduced by securing boundaries by fences or geographical features to prevent uncontrolled movement of livestock on and off the farm, maintaining contact with neighbours, inspecting and maintaining boundaries to ensure they remain secure, working with neighbours to limit the spread or impact of diseases, weeds and pests *(Moore and Payne, 2007)*.

The literature data emphasizes that dead animals can be a source of infection for other animals especially if they have died from an infectious disease. They also have the potential to cause offence to neighbours and to cause environmental damage. Steps to reduced the risks associated with dead animals might include a disposal plan for dead stock, engaging a veterinarian to investigate unusual outbreaks of disease and reduce the potential to compromise the biosecurity of the farm, a disposal areas that are secured and contained to prevent access by livestock, feral animals and wildlife, a disposal areas in locations that prevent nuisance to neighbours and spread of contaminants into waterways or the environment *(Hennessy, 2007; Maunsel and Donovan, 2008; Sibley, 2010)*.

Developing a dairy farm-level biosecurity plan

The initial step in a biosecurity plan is to assess goals and key concerns of the dairy farm. The producer along with advisors needs to determine just what infectious agents are important in their plan. Diseases such as Foot and Mouth Disease are so devastating for the entire farming community that state regulations and plans are in place to prevent such catastrophes *(Ellis-Iversen et al., 2011)*. Foreign animal diseases require a special set of biosecurity plans because they could potentially cover such a broad range of animal species and territory *(Henessy, 2007; Moore and Payne, 2007; Moore et al., 2008)*. However, on the individual farm level a variety of common domestic bacteria and viruses can be

identified as important disease problems and should be included in the biosecurity plan. On most dairy farms this would include diseases such as contagious mastitis, Johne's, *Salmonella spp*., BVD, Neospora, digital dermatitis, and a few others *(Henessy, 2007)*.

In most cases competent veterinarian works closely with the producer to develop and start the implementation of a biosecurity plan in the herd *(Moore and Payne, 2007; Gunn et al., 2008)*. However, each and every person who lives, works, or visits the farm has responsibility in the biosecurity plan. To make a biosecurity plan effective and easier to follow it is important to adopt practices that are customized to the individual farm setting and really make a difference. Not all animal groups are equally susceptible to infection and not all human activities are equally likely to contribute to disease control or potential spread. Strategies are being developed to help identify activities and animal groups that contribute to the risk of acquiring or preventing a specific disease. As part of that risk assessment, the level of risk can be categorized as low, medium, or high. Emphasizing high-risk animal groups and high-risk human activities can help to make the biosecurity plan more effective and simpler to implement. Cooperation and promotion of a sound biosecurity plan is important for the financial status of the farm, as well as the consumer through wholesome and high quality dairy products.

The following steps are typically involved in developing a farm-level biosecurity plan, as it is in Canada (*Anon., 2013*): complete a rational risk assessment to determine the disease problems, their magnitude, and the probability of occurrence, establish producer's goals for production and animal health, create a diagram of the dairy farm layout, identifying the production areas and animal-movement pathways, assign risk levels to the various production and management areas of the farm to determine the areas of greatest concern and vulnerability, identify the specific diseases of concern, determine the risk tolerance or intolerance to loss from infectious disease, outline the methods of prevention and control; and adopt the farm biosecurity program.

Any plan for preserving the required level of biosecurity should have three key components (*Uhlenhoop, 2007; Hristov et al., 2007; Anon., 2011*): 1. isolation and efficient separation and prevention contact between different age, production and geographically situated groups of cattle, 2. control the entry and movement on farm, indicating a clear overview and full control over the presence of foreign entities, personnel, foreign or other animals of the same species, vehicles, equipment, food and other items and materials in certain production segments on farm as a whole, and 3. sanitation, related to farm hygiene and any measures which reduce of pathogen agents in the production units and neighbouring farms.

Any biosecurity plan should provide all aspects about an appropriate location, breeding only one species in the production area, minimizing risks to the

cattle health (primarily from the environment and the production unit), additional biosecurity measures in the case of emergency of certain diseases, the hygienic-sanitary measures in all facilities and closest environment, imminent danger of an outbreak, keeping records on procurement animals introduction into production, morbidity, treatment, mortality, relocation and sales *(Anon. 2011)*.

The most aspects of farm-level biosecurity do not involve capital investment unless the infrastructure is poor. Indeed, many biosecurity programs can be developed solely on changes to current farm management practices and improved husbandry *(Plavšić et al., 2011)*.

It is important that producers review their production practices in relation to disease prevention on a frequent basis and ensure that their strategies are being carried out. An effective farm-level biosecurity plan should remain flexible and open to new scientific knowledge and technology as they become available *(Kristensen and Jakobsen, 2011)*.

In the past, much of the emphasis on biosecurity has been focused on animals and infectious disease agent(s). An important component of biosecurity that often has been overlooked is the influence of the dairy farm environment. Facility design, layout, and traffic patterns can significantly affect the spread or prevention of disease and have significant influence on the effectiveness and efficiency of a farm-level biosecurity plan *(Brennan and Christley, 2012)*.

The concept of setting up a controlled access zone, transition points, and a restricted access zone is being adopted worldwide (*Wolfgang et al., 2003; Anon., 2013*). The reasons for establishing the zones are to protect the dairy herd from contamination carried in by outside agents (animals, people, vehicles, equipment, feed, water, and pests) that enter a zone and to manage and contain any disease issues within the herd.

Conclusion

According to all previously presented data concerning dairy farm risk, assessment and development of biosecurity plan in dairy farm production, it might be concluded that:

- dairy cattle farm biosecurity refers to all management practices that reduce the chances infectious and parasitic diseases will be carried onto the farm by animals, people, vehicles, equipment, feedstuff, bedding materials, effluent and waste materials and also spread infectious and parasitic diseases on the farm;
- key responsibility for dairy farm-level biosecurity belongs to the producer or herd owner, veterinarian, employees and visitors ;

- the process of evaluating a farm to determine its potential risks of entry or spread of disease and to identify practical methods of limiting or mitigating these risks is the foundation for developing a systematic biosecurity plan;
- successful biosecurity plan must address isolation of new animals brought to the farm, isolation of sick animals, regulation of the movement of people, animals, and equipment, and procedures for cleaning and disinfecting facilities;
- key elements of a herd health program include vaccinations, comprehensive mastitis prevention program, work with neighbours to minimise spread of diseases, reporting unusual outbreaks of disease, and plan for prompt disposal of dead stock.

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Procena rizika na farmi muznih krava i izrada plana biosigurnosti

S. Hristov, B. Stanković, N. Maksimović, D. Ostojić-Andrić, Z. Zlatanović

Rezime

U radu su opisana najznačajnija gledišta u pogledu procene rizika i izrade plana biosigurnosti u farmskoj proizvodnji muznih krava. Procena rizika podrazumeva način utvrđivanja prisustva, širenja i uticaja neke infektivne bolesti po farmsku proizvodnju. Nakon definisanja područja rizika, moguće je primeniti odgovarajuće mere za kontrolu bolesti. Mogućnost unošenja infektivnih ili parazitskih agenasa u stado se može umanjiti kroz pažljivu procenu zdravstvenog stanja u pogledu ovih oboljenja, pregledom novonabavljenih grla pre uvođenja u stado tokom izolacije, nabavkom semena i embriona isključivo od proverenih dobavljača, kao i uvidom u zdravstvena uverenja životinja koje uvode ili se vraćaju u stado, čime se stiče uvid i kontrola nad brojem kontakata sa drugim životinjama.

Svaki plan biosigurnosti mora da uzme u obzir sve aspekte u pogledu lokacije na kojoj se farmska proizvodnja obavlja i zastupljenosti samo jedne životinjske vrste na njoj, čime se na najmanju meru svodi rizik po zdravlje muznih krava, kada su u pitanju agensi iz okruženja ili sa same farme, uz primenu dodatnih

biosigurnosnih mera u slučaju pojave infektivnih oboljenja, higijensko-sanitarnih mera u svim segmentima proizvodnje i bliskom okruženju u slučaju neposredne opasnosti od izbijanja bolesti, uz vođenje evidencije o uvođenju životinja u proizvodnju, morbiditetu, tretiranju obolelih, mortalitetu, preseljenju i prodaji životinja.

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A SURVEY OF BEEF CARCASS AND QUALITY TRAITS IN SLOVAKIA

I. Bahelka, M. Gondeková

Animal Production Research Centre, Hlohovecká 2, 951 41 Nitra-Lužianky, Slovak Republic
Corresponding author: bahelka@cvzv.sk
Invited paper

Abstract: The objective of the present study was to evaluate carcass and beef quality traits in cattle population of Slovakia. Eight slaughterhouses located in the western, central and eastern part of Slovakia were surveyed to provide nationwide representation. The study was performed from November 2010 to June 2012. The sample of the loin (1000 g) between $9^{th}/11^{th}$ rib was removed from the each carcass within forty-eight hours after slaughter. Totally, 300 samples were collected, of which were 181 cows, 78 bulls and 41 heifers. The most represented were Holstein (34.7 %), Slovak Pied (17.0 %), beef breeds (11.0 %) and Pinzgau breed (8.7 %). Average live weight of the cattle was 470.0 kg at age of 1 924 days (5.25 years). According to European beef carcass grading standards, average carcass fatness was 1.9 corresponding with thin layer of subcutaneous fat. Carcass conformation varied from poor (class P) to good (class R) in all cases. The best carcass conformation was found in bulls, slightly lower in heifers and these two categories differed significantly from cows. Significant differences were analysed between sex in chemical composition. Bulls had the highest total water content (75.56 % vs. 74.60 and 73.76 %). On the other hand, cows and heifers had significantly higher content of intramuscular fat than bulls (3.54 and 3.49 % vs. 1.98 %) what resulted in differences in energetic value of meat. Only 5 samples (1.6 %) of carcasses exhibited parameters of DFD and DCB beef, resp. As expected, meat of cows was significantly darker than that of two others categories, and together with meat of heifers had higher marbling than beef of bulls (7.92 and 7.90 vs. 8.50). Sensory properties were evaluated by consumer test on the 7^{th} day after slaughter. There were not any differences in tenderness and juiciness between bulls, cows and heifers. Only flavour and odour of meat were evaluated significantly better in bulls than cows. Information from this survey provide data that could be use for supporting the cattle breeding and beef consumption in Slovak Republic.

Key words: beef, survey, carcass and quality traits

Introduction

Cattle breeding and beef production has a long tradition in Slovakia. Beef as an abundant resource of essential amino acids, vitamins, especially of group B and minerals, mainly hem iron represents significant component of the nourishment of people. Despite all these positives, beef consumption in Slovakia has been continually decreasing. While in 1990 it was 22.8 kg per capita and comparable with West Europe, at present represents only 4.9 kg which seems to be strange comparing to other EU countries and worldwide. A drop of beef consumption nowadays is not a short-term issue but it is the result of our previous efforts – to prefer the cattle breeding without feedback and consumer´s demand. Actually, this condition is caused by several factors. One of them and, perhaps, the main reason is relatively high price of beef due to expensive production costs. Beef has been becoming a „luxurious" food accessible to wealthier strata of population. Recent studies *(Mojto et al., 2000; Zaujec et al., 2006; Zaujec and Gondeková, 2011*) indicate the increasing share (up to 50 %) of cows from total number of cattle slaughtering in Slovakia. The causes of culling are different such as health reasons, reproductive problems, low milk performamce, etc. Hence for slaughter purpose, cows culled from the breeding are used. Since the most of these cows are from dairy farms focused on milk not meat production, slovak consumer can perceive meat of cows and its quality as something inferior, subordinate, just by-product of milk production. Moreover, cow´s meat is traditionally considered as dark, firm, less juicy and tender and therefore less valuable and not attractive for consumer. The aim of the study was to obtain actual picture of the carcass and meat quality of beef produced and consumed under condition of Slovak Republic.

Material and Methods

Eight slaughterhouses located in the western, central and eastern part of Slovakia were surveyed to provide nationwide representation. The study was performed from November 2010 to June 2012. The sample of the loin (800 - 1000 g) between $9^{th}/11^{th}$ rib was removed from the each carcass within forty-eight hours after slaughter. Overall, 300 samples were collected, of which were cows (n = 181) , bulls (n = 78) and heifers (n = 41). The most represented were Holstein (34.7 %), Slovak Pied (17.0 %), beef breeds (11.0 %), Pinzgau (8.7 %) and crosses of Slovak Pied (13.0 %).

When samples were taken, data of each animal were recorded as follows: identification number, data of birth and slaughtering, age at slaughter, live and carcass weight, carcass fatness and conformation according to SEUROP system.

Analyses of meat samples were performed in the laboratory of Animal Production Research Centre Nitra 48 hours after slaughter. Chemical composition of meat was determined using device INFRATEC 1265. Electrical conductivity (μS) was analysed by device BIOTECH and pH values were determined by apparatus METTLER TOLEDO with combined electrode. Meat colour was analysed by spectrometry using device MINISCAN XE Plus. Water holding capacity (WHC) was analysed using method of Grau-Hamm modified by *Hašek and Palanská (1976)*. Drip loss of meat was determined by method of *Honikel (1986).* Marbling of the samples was measured using 10-point scale *(USDA, 1997)* where 1 – the highest and 10 – no visible intramuscular fat.

Seven days after slaughter, i.e. after aging of meat, cooking loss, pH and shear force were determined. Shear force – firmness was measured using device TEXTURE ANALYSER TA-XT2i. Before cooking, 2.0 cm thick slices of *m. longissimus dorsi* were cutting. After that they were cooking on electric contact grill PM-1015 for 4 min at t = 200 °C and cooking loss was determined as difference between weight of meat before and after cooking. Consequently the sensory properties of meat such as odour, taste, juiciness and tenderness were rated using consumer test. Every sample was evaluated by three persons using 5-point scale where 1 – the worst and 5 – the best quality.

Data were analysed by statistical package SAS 9.2. Basic statistics was done using MEANS procedure.

Results and Discussion

Average slaughter weight of cattle was almost 470.0 kg at age of 1 924 days, i.e. 5 years and 3 months (table 1). That is in agreement with results of *Méndez et al. (2009)* who found in the survey in Mexico that 71.6 % of cattle had slaughter weight in the range between 400 and 500 kg. Lower slaughter weights were presented in another study from Mexico *(Martínez et al., 2006)* and Costa Rica *(ITCR, 2004)*. When carcass quality was evaluated, average fatness of carcasses represented only low level (1.90), i.e. thin layer of subcutaneous fat with markedly visible lean meat. For evaluation the carcass conformation, numeric scale 1-6 was used where 1 corresponded to the class P and 6 to class S according to SEUROP system. Average value of 1.71 represented below-average up to medium conformation. No animal evaluated in this study did not reach higher class than R for carcass conformation. This is rather surprising finding since purebred beef bulls or crosses of this cattle with Slovak Pied were included in the study. On the other hand, our finding corresponds with results of some previous studies *(Mojto et al., 2000; Zaujec et al., 2006; Zaujec and Gondeková, 2011*). Higher carcass conformation (3.0) was published by *Méndez et al. (2009)*.

Table 1. Carcass parameters of the cattle (n = 300)

Trait	$\bar{x}$	sd
EUROP – conformation	1.71	0.59
EUROP – fatness	1.90	0.85
Carcass weight, kg	246.05	83.98
Slaughter weight, kg	468.88	158.67
Age at slaughter, days	1924.02	1359.72

Chemical composition of meat is shown in table 2. The values presented are typical for beef and comparable with other studies *(Gondeková et al., 2008, Zaujec et al., 2010).*

Table 2. Chemical composition of meat of the cattle (n = 300)

Trait	$\bar{x}$	sd
Total water, %	74.74	3.49
Total protein, %	21.13	1.19
Intramuscular fat, %	3.13	3.35
Energetic value, kJ/100g	471.84	126.87

Meat quality parameters of whole cattle set are shown in table 3. Analysis of pH found that only five samples shared values over 6.2, what is considered signs of DFD or DCB meat. Marbling had average value of 8 which is slight value. Similar levels of marbling characterised as „slight, practically devoid" or „traces" have been published by *Méndez et al. (2009)* and *Rubio et al. (2007).* In the survey of the beef quality in the USA, *McKenna et al. (2002)* classified 76.6 % of cattle carcasses as small or slight marbling.

Table 3. Meat quality of the cattle (n = 300)

Trait	$\bar{x}$	sd
pH_{48}	5.62	0.33
pH_7	5.69	0.35
Elektrical conductivity, µS	2.05	1.48
Colour - L	29.91	3.51
a	11.42	2.75
b	7.29	1.50
Water holding capacity, %	28.28	6.78
Cooking loss, %	26.80	7.39
Firmness, W-B	10.16	4.31
Drip loss, %	1.19	0.50
Marbling	8.07	1.36

From sensory point of view, the best evaluation in our study was assigned to odour of meat followed by taste, juiciness and tenderness (table 4).

Table 4. Senzory parameters of meat (n = 300)

Trait	$\bar{x}$	sd
Taste	3.37	0.73
Odour	3.59	0.68
Tenderness	3.19	0.90
Juiciness	3.23	0.74

After splitting whole set into categories of bulls, cows and heifers, the best carcass conformation was found in bulls and only slightly lower in heifers. The difference between these two groups and category of cows was statistically significant (table 5). Bulls were slaughtered at the highest live weight and the lowest age at slaughter. It is in agreement with results of *Méndez et al. (2009)*.

Table 5. Comparison of carcass quality between categories of the cattle

Trait	Cows (n = 181)		Bulls (n = 78)		Heifers (n = 41)	
	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd
EUROP – conformation	1.56[a]	0.57	1.97[b]	0.54	1.88[b]	0.56
EUROP – fatness	1.89	0.92	1.83	0.38	2.07	1.13
Carcass weight, kg	233.87[a]	86.88	279.12[b]	66.21	238.18[a]	85.97
Slaughter weight, kg	453.70[a]	168.55	508.53[b]	120.72	462.08	166.80
Age at slaughter, days	2682.00[a]	1278.89	741.00[b]	167.84	975.00[c]	374.29

[a,b,c] P<0,05 min.

Significant differences were determined in chemical composition of meat between categories (table 6.) Bulls had significantly highest total water content, since cows and heifers had higher content of intramuscular fat and energy value of meat than bulls. Even intramuscular fat content of bulls was under recommended value if taken into account sensory aspects for beef (2.5 – 3.0 %). As known, deposition of intramuscular fat in the muscles is influenced by age and gender of the animals. Individual females (cows, heifers) have physiologically different deposition of intramuscular fat than males – bulls, which is expression of different metabolism controlled by sex hormones.

Table 6. Comparison of chemical composition of meat between categories of the cattle

Trait	Cows (n = 181)		Bulls (n = 78)		Heifers (n = 41)	
	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd
Total water, %	74.60[a]	4.00	75.56[b]	1.82	73.76[a]	3.19
Total protein, %	20.86[a]	1.28	21.46[b]	0.78	21.75[b]	1.06
Intramuscular fat, %	3.54[a]	3.83	1.98[b]	1.74	3.49[a]	2.95
Energetic value, kJ/100g	482.69[a]	145.54	434.13[b]	65.28	495.71[a]	113.62

[a,b] P<0,05 min.

Meat quality traits regarding to cattle categories are shown in table 7. Heifers reached significantly lower values of pH after 48 hours or 7 days in comparison to cows and bulls. However, the values are considered to be normal in the all cases. Significant differences between bulls, cows and heifers each other were found in electrical conductivity. The darkest meat was determined in cows and it was significantly different from meat colour of bulls and heifers. Drip loss was very low in all three categories but in heifers were statistically lower than in other two groups. Cows and heifers reached actually consistent marbling which was significantly higher than that of bulls. There is an evident linkage with laboratory analysed intramuscular fat content. Slightly higher but not significant result in favor of heifers over bulls found *Méndez et al. (2009)*. From consumer´s point of view the best evaluation of sensory properties was achieved in category of bulls and the lowest in cows. However, only odour and taste differed significantly within both groups. The values of heifers have been kept between category of bulls and cows (table 8). *Beriain et al. (2009)* evaluated two types of beef in Spain – from yearling Pyrenean bulls and US beef originated from beef cattle slaughtered in EU. Taste of beef from spanish bulls was evaluated worse (3.14-3.42) than that of bulls in our study, while US beef better (3.46-3.67). Similar result was found in tenderness and juiciness, while odour of both types of beef was under our value.

Table 7. Comparison of meat quality between categories of the cattle

Trait	Cows (n = 181)		Bulls (n = 78)		Heifers (n = 41)	
	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd
pH_{48}	5.73[a]	0.42	5.59[a]	0.26	5.40[b]	0.17
pH_7	5.69[a]	0.32	5.79[a]	0.44	5.56[b]	0.30
Elektrical conductivity, µS	2.12[a]	1.48	1.29[b]	0.96	3.15[c]	1.54
Colour - L	29.48[a]	3.59	30.43[b]	3.00	30.81[b]	3.84
a	1.84[a]	2.84	10.65[b]	2.26	11.01[b]	2.87
b	7.27	1.58	7.39	1.37	7.16	1.41
Water holding capacity, %	28.01	6.88	28.13	6.69	29.77	6.50
Cooking loss, %	27.20	7.31	26.42	7.55	25.70	7.49
Firmness, W-B	10.13	3.90	10.34	4.94	9.98	4.85
Drip loss, %	1.20[a]	0.43	1.24[a]	0.67	1.05[b]	0.43
Marbling	7.92[a]	1.50	8.50[b]	0.91	7.90[a]	1.32

[a,b,c] P<0,05 min.

Table 8. Comparison of senzory properties between categories of the cattle

Trait	Cows (n = 181)		Bulls (n = 78)		Heifers (n = 41)	
	$\bar{x}$	sd	$\bar{x}$	sd	$\bar{x}$	sd
Taste	3.30[a]	0.75	3.53[b]	0.67	3.39	0.71
Odour	3.51[a]	0.73	3.78[b]	0.61	3.60	0.53
Tenderness	3.13	0.83	3.30	1.07	3.22	0.86
Juiciness	3.19	0.72	3.36	0.82	3.14	0.63

[a,b,c] P<0,05 min.

Conclusion

Carcass quality of cattle produced under slovak condition is quite low and does not reach the level of production abroad. The causes must be sought mainly in economics of beef production. On the other hand, meat quality parameters were at a good level, only 1.7 % of samples showed signs of DFD or DCB meat. As expected, meat of cows was significantly darker than that of two others categories, and together with meat of heifers had higher marbling than meat of bulls. However, marbling of beef in slovak cattle population is on the low level. The study did not confirm expected essential differences between meat of bulls and cows. The results obtained should contribute to the knowledge of the carcass and beef quality as well as consumer´s preferences in order to promote the cattle breeding and beef consumption in Slovakia.

Ispitivanje kvaliteta i osobina junećih trupova u Slovačkoj

I. Bahelka, M. Gondekova

Rezime

Uzgoj stoke i govedarstvo imaju dugu tradiciju u Slovačkoj. Dok je potrošnja govedine u 1990. bila 22,8 kg po glavi stanovnika i uporediva sa Zapadnom Evropom, u ovom trenutku predstavlja samo 4,9 kg što izgleda čudno u odnosu na ostale zemlje EU i sveta. Nedavne studije ukazuju na sve veći deo (do 50%) krava od ukupnog broja goveda za klanje u Slovačkoj. Budući da većina ovih krava potiče sa farmi muznih grla koje su fokusirane na mleko, a ne na meso, slovački potrošač može smatrati da je meso krava i njegov kvalitet nešto lošiji, samo kao nusproizvod u proizvodnji mleka.

Cilj studije je bio da se dobije stvarna slika o trupu i kvalitetu junećeg mesa proizvedenog i konzumiranog u uslovima Republike Slovačke. Osam klanica koje se nalaze u zapadnom, centralnom i istočnom delu Slovačke, je ispitano kako bi se obezbedila nacionalna zastupljenost. Studija je sprovedena od novembra 2010 do juna 2012. Uzorak slabine (800 - 1000 g) između 9./11. rebra je uklonjen sa svakog trupa u roku od četrdeset osam sati nakon klanja. Ukupno 300 uzoraka je prikupljeno, od krava (n = 181), bikova (n = 78) i junica (n = 41). Najzastupljeniji su bili holštajn (34,7%), slovački pied (17,0%), tovne rase (11,0%), pincgau (8,7%) i melezi slovačke pied rase (13,0%).

Analize hemijskog sastava i kvaliteta mesa su vršene u laboratoriji za stočarstvo Istraživačkog centra u Nitri 48 časova posle klanja. Sedam dana nakon klanja su određivane senzorne osobine mesa.

Prosečna težina goveda pred klanje bila je gotovo 470,0 kg u uzrastu od 1.924 dana, odnosno 5 godina i 3 meseca. Debljina trupova je bila na niskom nivou (1,90), a konformacija trupa ispod prosečnog do srednjeg nivoa (1,71) što ukazuje na lošiji kvalitet trupa stoke proizvedenih u Slovačkoj u poređenju sa zemljama Zapadne Evrope. Značajne razlike u hemijskom sastavu su analizirane između životinja različitog pola. Bikovi su imali najveći ukupan sadržaj vode u odnosu na krave i junice (75,56% prema 74,60 i 73,76%). S druge strane, krave i junice su imale veći sadržaj intramuskularne masti od bikova (3,54 i 3,49% prema 1,98%) što je dovelo do razlika u energetskoj vrednost mesa. Samo 5 uzoraka (1,6%) trupova je imalo parametre DFD i DCB junećeg mesa. Kao što se očekivalo, kravlje meso je znatno tamnije nego kod druge dve kategorije, i zajedno sa mesom junica ima bolju marmoriranost, u poređenju sa mesom bikova (7,92 i 7,90 prema 8,50). Sa stanovišta gledišta potrošača, najbolja ocena senzornih svojstava

ostvarena je u kategoriji bikova, a najniža u kategoriji krava. Međutim, samo su se miris i ukus značajno razlikovali u obe grupe.
Informacije iz ovog istraživanja daju podatke koji se mogu iskoristiti za podršku uzgoju stoke i potrošnje govedine u Slovačkoj Republici.

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CULINARY VALUES OF BEEF MEAT

W. Migdał[1], B. Živković[2], J. Tkaczewska[1], P. Kulawik[1], A. Migdał[3], Ł. Migdał[4]

[1]Department of Animal Product Technology, Faculty of Food Technology, University of Agriculture in Krakow, Balicka 122, 31-149 Kraków, Poland
[2]Institute for Animal Husbandry, Autoput 16, 11080 Zemun, Republic of Serbia
[3] Department of Animal Reproduction and Anatomy, University of Agriculture in Krakow, Al. Mickiewicza 24/28, 30-059 Krakow, Poland
[4]Department of Poultry and Fur Animal Breeding and Animal Hygiene, University of Agriculture in Krakow, al. Mickiewicza 24/28, 30-059 Kraków, Poland
Corresponding author: w.migdal@ur.krakow.pl
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Abstract: Beef and veal consumption quality is characterized by esthetic look, flavor, tenderness, succulence. Nutritional quality is characterized by content and composition of proteins, fats, vitamins, mineral components and digestibility. Technological quality is the water holding capacity, pH, fat, protein and connective tissue content and the size of meat pieces. The sanitary quality depends on the amount of microorganisms, residues of harmful substances and contaminations. The ethical quality depends on organic farming, religious believes as well as welfare aspects. The increase in prices results also from improved quality features of beef. Beef belongs to one of the most valuable meats in regard to nutrition value, which is determined by the proximate composition and the content of exogenous compounds.

Key words: beef meat, culinary value

Beef is a valuable meat with very good culinary value. High nutritional quality, its functional properties and the tradition of beef consumption in Poland are the main causes that beef is very popular among the consumers. Although it belongs to the culinary meats, the high quality and high price beef hams should not be forgotten. Mature beef is a meat of the highest flavor values, which can be used for boiling, frying, cooking, grilling and braising. It is the ingredient for preparation of many different meals. Despite all that, culinary beef in its true meaning (labeled according to anatomical sections of carcass) can only be found in special brand stores. In regular meat store, the beef is of poor quality, since it is usually acquired from defective dairy cows or from fattening of small bulls derived from dairy cows. In Poland most of produced beef comes from the average quality

livestock (mostly from farms oriented towards milk production, while meat is a secondary product) (*Stańko end Lewandowski, 2007*).
However there is an increase in the amount of beef characterized by good quality and acquired from typical meat breeds. High quality meat, acquired from meat-specialized breeds of cattle has its price and is consumed by a demanding customer, which is why the increase in beef production should be accompanied by the change in the production structure, meaning increase in market share of high quality beef acquired from cattle of meat breeds and meat-dairy crossbreeds. Such changes should be supported by the integration of cattle livestock producers with meat industry, because meat industry possesses means (the price of the meat) to force such changes. This would be an important factor influencing the growth of Polish cattle population and increase in production of high quality beef both for the domestic market and for export, especially since Polish beef is recognized by foreign customers, who remember that Polish cattle was hardly contaminated by the BSE epidemic (only 76 cases of BSE in Poland). The system of labeling and culinary nomenclature of beef and veal requires immediate and absolute implementation. The schemes of beef and veal carcass with culinary nomenclature of elements along with their culinary designation (for roast, grill or broth) will allow distinguishing the basic meat quality standards, culinary nomenclature and will cause the demand for specific carcass elements. But one of the most important features is the best quality of beef and veal, and its quality is not limited only to fat content, color and scent. The answer to all above problems, for both consumers and breeders alike, can be the Quality Meat Program System (QMP), the first Polish system of guaranteed meat quality, developed and implemented by the organizations of agricultural producers. The owner of the QMP system is Polskie Zrzeszenie Producentów Bydła Mięsnego. System, which is both open and voluntary, gathers farmers, small and middle enterprises including groups of producers of bovine livestock and beef, slaughterhouses, processing plants and the individual participants of the beef distribution chain.

Polish Association of Beef Cattle Producers (Polskie Zrzeszenie Producentów Bydła Mięsnego - PZPBM) signed in 2007 an agreement called Eurostek 2012. Under those regulations PZPBM developes and implements the high quality beef production program called Eurostek 2012, whose goal is to increase the beef produced under quality control systems to the level of 30 000 tones in 2012. The QPM quality system is the main operation of the Eurostek 2012 program.

Opportunities for Polish beef industry are enterprises like Warmińsko – Mazurski Klaster Wołowy – union of producers, breeders, slaughterhouse owners, processing units and retail chain distributors. They all stride to achieve one goal – to promote the high quality beef from Warmińsko - Mazurskie region of Poland. The purpose of this cluster is also the development of cooperation chain and building interpersonal relationships, supporting the expansion of companies operating within the cluster, establishing the web of connections between the companies, enabling the implementation of innovative solutions, luring new interesting companies and young talents into the region, crating the recognizable brand of the region, promotion of export from the cluster area, market intelligence collection, distribution of new technologies within the cluster, improvement of production processes, lobbying in favor for the development of infrastructure, coordination of purchases within the cluster, etc. Beef cluster is a brand which will be a warrantor of beef possessing unique flavor values and perfect texture parameters. The beef cluster, in their own retail chain, offers diverse assortment of products including the most precious meat parts like beef sirloin. The benefits for participants of the cluster are: lower transportation costs, lower transaction costs, lack of development barrier, higher productivity, possibility for cooperation and specialization and higher quality of products.

When taken into consideration that most of the beef and veal is delivered for direct consumption, the consumers demands are believed to be the most important, with regard towards their experiences and habits. The processed beef meats should not be forgotten however. High quality beef is a semi-caloric meat, leaner then pork. With current nutritional tendencies towards reduction of energetic value of food, this factor plays a very important role. Moreover beef contains significant amounts of proteins of high biological values. Meat is an essential part of human diet due to unique chemical composition, nutritional value, the content of protein with beneficial amino acids composition and functional properties (*Bender, 1992*). Proteins are the main ingredient of muscle tissue and on average make up for 15-24% mass of muscle tissue of slaughter animals. The beef protein contains all exogenous amino acids, necessary for synthesis of body proteins (phenylalanine, isoleucine, leucine, valine, methionine, threonine, tryptophan, and lysine). Proteins of connective tissue (collagen and elastin) have lower biological value. Meat proteins, present in cell determine the structure and physicochemical

properties of tissues such as resilience, flexibility, etc. Daily intake of adult for protein should be around 0,8-1g per 1 kg of body weight, so the demand for protein of an adult weighing 70 kg is 56-70 g of protein daily. 100 g serving of lean meat provides around 20 g of protein, which is 28-36% of the daily demand for this nutrient (*Kunachowicz et al., 2000)*. Non-protein nitrogen compounds (which constitute around 15% of total nitrogen content in meat) are soluble in water compounds, without the properties of proteins and influencing the utility and sensory value of meat. They give the meat and its broth the specific taste and scent. This includes such compounds as: creatine, creatinine, carnosine, arginine phosphoric acid, creatine phosphoric acid and purine derivatives. Carbohydrates constitute of around 1% (0,5-1,5%) of muscle weigh. The main muscle carbohydrate is glycogen – compound build from glucose molecules. Different animal tissues contain from 0,3 till 1,3% of glycogen. Liver, which is especially rich in glycogen, can contain around 3% of this carbohydrate. Glycogen is the reserve of glucose, which is used as the energy source in tissue metabolism of the body such as glycolysis, during the muscle contraction or during the ripening of meat. Moreover meat contains high amounts of free sugars, such as glucose, fructose, mannose and the products of their conversion like organic acids – lactic, pyruvic or oxaloacetic acids. The mineral nutrients present in meat are mainly potassium, sodium, calcium, magnesium, iron, phosphorus, silicon, chloride and micronutrients. Beef is a rich source of iron, necessary for the synthesis of hemoglobin, and zinc, selenium and copper which are parts of different enzymes. In the highest amounts, meat contains potassium, phosphorus, magnesium, iron, and little calcium (with the exception of offal). The highest amounts of iron are present in beef and veal meat (1,8-3 mg per 100 g). Beef contains on average 2,5 times more iron then pork meat, and 5 times more than poultry (0,4 mg/100g). Human daily recommendations for iron, ranges depending on sex and age, from 10-26 mg, so 100 g of beef covers from 9-33% of human daily demand for iron (*Makarewicz-Wujec and Kozłowska-Wojciechowska, 1999*). Poultry covers just 1,5-4% of daily demand for this compound. There are two types of iron in food products: easily absorbable hemic iron (contained in animal products) and hard to absorb non-hemic iron (contained in plant products). Iron is the best absorbable from meals containing meat, fish and vitamin C. The iron absorption from cereal products and vegetables is low, but it can be enhanced by the addition of vitamin C or meat into the meal. In animal products around 40-50% of iron present occurs in easily absorbable hemic form. During the digestion process, however, usually half of it is lost and the documented absorption of hemic iron from food products is around 20%. Because around 75% of daily food rations consist of plant products, in which the iron is present in hard to absorb non-hemic form, the total iron absorption of iron from typical human diet is around 10%. The absorption of iron

from rice is 1%, corn 3%, lettuce 4%, wheat 5%, fish 11%, liver 12-14% and veal meat around 22%. Through skillful combination of meat products with vegetables, cereals and legumes the absorption of non-hemic iron can be enhanced in easy and effective way. This enhancement is called the "meat factor" in the meat of mammals, poultry and fish (*Makarewicz-Wujec and Kozłowska-Wojciechowska, 1999*). The highest zinc amounts are present in beef (4,6mg/100g), which contains few times more zinc than other meats. 100 g serving of beef covers 20 – 30% of daily recommendations for zinc. The increased demand for zinc occurs during pregnancy and lactation (*Cichocka, 2005*). Moreover meat contains high amounts of copper, aluminum, magnesium, cobalt, iodine and other trace elements. The important beef meat feature is high content of vitamins from group B, vitamins A, D, E and bioactive substances with antioxidative functions such as carnosine and glutathione. The B_2 vitamin is present in both veal and beef at the level of around 0,2mg/100g. The turkey and chicken meat contains around 0,15mg/100g and the amount of this vitamin in fish varies from 0,04 till 0,3mg/100g. 100g serving of beef covers around 10% of daily demand of women and 5% of daily demand of man for this vitamin. Veal is a rich source of PP vitamin (niacin) and contains around 16mg of this vitamin in 100g. The 100g serving of veal covers around 50% of daily recommendations of both men and women for niacin (*Cichocka, 2005; Kunachowicz et al., 2000*). Meat is the main source of vitamin B_{12}. The 100g serving of beef or veal contains 1,4-2,5 μg of vitamin B_{12} and covers on average half of the daily demand. Vitamin B_{12} is necessary for the growth of all human body cells. Along with folic acid it takes part in DNA synthesis and in carbohydrate and lipid metabolism. The lack of this vitamin can cause anemia. Daily demand for this vitamin is 2 μg for children age 1-3, 3 μg for older children and 3-4 μg for adults. The 100g serving of meat covers 17-85% demand for vitamin B_{12}, depending on the type of meat and individual requirements for each human (*Cichocka, 2005; Makarewicz-Wujec and Kozłowska-Wojciechowska, 1999*). Moreover meat contains thiamine and pantothenic acid. Animal fat is also the source of fat soluble vitamins: A, D and E. Yellow color of fat tissue of some animals (especially those fed with corn), is the result of accumulation of carotenoids. The β-caroten content in beef can reach even 10 μg. Often the meat is accused of containing fat, especially the high amount of saturated fatty acids and cholesterol and thereby contributing towards atherosclerosis. Beef is the meat of diverse energy value because the amount of muscular fat can vary between 1,5% up to even 13%. Animal fat are built from mixed triacyloglycerols, phospholipids, mono and diacyloglycerols and free fatty acids. The most commonly encountered in meat phospholipids include lecithin (phosphatidylocholine) and cefaline (phosphatydyloetanolamine). The amount of fat depends on the animal species and degree of fattening. The highest amount of fat contain very fatty pork, beef and

mutton (not to be mistaken with delicate and lean lamb), and the lowest – veal and ostrich meat. In the carcass the fat is present as either subcutaneous fat, internal fat (surrounding the internal organs – perirenal, intra-abdominal), intramuscular fat and intermuscular fat. Fat tissue accumulated in muscle stroma is responsible for so called marbling. High fluctuations in the fat content depend mostly on the type of muscle and the degree of fattening. Acquisition of optimal intramuscular fat is crucial fat is closely correlated with many sensory qualities of meat – texture and flavor. Intramuscular fat of beef contains almost 44% of saturated fatty acids (SFA), 46% of monounsaturated fatty acids (MUFA) and 10% of polyunsaturated fatty acids (PUFA), including the conjugated dienes of linoleic acid – CLA. The beef fat is characterized with lower content of PUFA (*Kołczak, 2008*) when compared to plant fats or fish oils and higher cholesterol content. A common practice is to modify the fatty acids profile of the fat of the slaughter animals (including cattle) by feeding modification, but too high content of PUFA in inintramuscular or reserve fat influences unfavorably the technological and quality properties of meat – mostly sensory properties. Soft, greasy, fatty taste and scent deterioration, lowered stability and limited storage methods are the most important problems related to the modification of fatty acids profile of meat and its products. The changes related to the fat oxidation in muscle tissue are the main cause of the undesirable chemical and sensory changes of not only the meat as a resource but also its processed products, which is why the addition of PUFA in fodder for livestock requires the usage of antioxidants like vitamin E (tocopherol). Moreover the addition of fat to the fodder of ruminants may be deadly for the rumen microflora and requires the use of so called "protected fat". The share of CLA and PUFA in meat processed for long-term high thermal treatment, may lead to creation of trans isomers, which affect unfavorably many biochemical and physiological processes in human body. The fatty acids, by supplying the aldehydes, take part in synthesis of chemical compounds, built from heterocyclic rings containing sulfur and/or nitrogen, which give the typical meat aroma. Those compounds include lactams, alkylfurans, alkylpirydynes and alkylthiazoles. The most important aldehydes, giving the undesirable odor, are hexanal and pentanal. Many of those lipid oxidation products are formed during the thermal treatment – frying, grilling or boiling. Linoleic acid (C18:2) instantly oxidizes during boiling, which results in distinctive rancid odor of meat products. The main product of arachidonic acid (C20:4) oxidation is 1-octen-3-ol, which results in a fungal odor of meat products. As a result of oxidation of n-3 fatty acids, the meat acquires an unacceptable for consumer fishy odor. The carriers of this odor are: α-linolenic acid (C18:3), eicosapentaenoic acid (C20:5) and docosahexaenoic acid (C22:6). American and British studies showed, that the upper acceptability limit of content of those acids (the sum of C18:3 + C20:5 + C22:6) for the consumers is 3% of all

the fatty acids. At the same time, the comparative studies showed, that the meat containing more n-6 fatty acids is more flavorful then the meat containing more n-3 fatty acids (*Wood, 1984; Wood et al., 1994*). Stearic acid is crucial for the development of meat tenderness and succulence. Besides the overall content of fatty acid groups with different degree of saturation, other important marker of fat quality is the ratio of unsaturated fatty acids (UFA) to the SFA, which in human diet should be around 2. This ratio is the most preferable in veal meat. In lean beef, the ratio of PUFA to SFA is around 0,5-0,7 and is close to the amount recommended by dieticians. The study performed by *Wood et al. (1999, 2004)* showed the positive correlation between flavor of meat and the content of SFA and MUFA, and negative correlation to PUFA content. In normal daily food ration, the lipids should provide around 20-35% of energy, at the same time covering the demand for fat soluble vitamins and essential fatty acids (EFF). The ratio between vitamin E (mg) to EFF (g) should be at least 0,6 (Harris coefficient). According to EFSA (European Food Safety Authority) daily demand for n-3 fatty acids is 2,2g. Oleic acid (C18:1) shows the positive effect in atherosclerosis prevention. The sum of lauric (C12:0) and myristic (C14:0) acids, the acids with hypercholesterolemic effect in beef's fat, is around 2-3,7%. The high content of SFA contributes to formation of negative opinions in regard to beefs nutritional value. It should be stated however that not all SFA show the adverse biological effect. Palmitic acid (C16:0) and present in low amounts myristic acid increase the LDL fraction of blood cholesterol, while stearic acid is considered to be neutral. Negative impact is also not shown by the, dominant if beef meat, MUFA. The PUFA/SFA ratio is widely accepted to be the marker of fat quality, in terms of human health, but favorable PUFA/SFA ratio is not necessarily correlated with the beneficial n-6/n-3 ratio, because the increase in PUFA is usually caused by the increase in n-6 fatty acids. Ulbricht and Southgate *(1991)* proposed different indicators of fat quality, such as the atherogencity index (AI). It establishes the ratio of SFA (myristic and palmitic acids) to UFA (PUFA + MUFA), indicating the significant negative role of myristic acids and positive role of MUFA in human nutrition. AI is the most preferable in veal (0,312), while the least preferable are rabbit and fattening cattle meats. The veal and goat meat, showed to have the most valuable ratio of the hypo and hypercholesterolemic fatty acids. It is believed that the hypocholesterolemic acids decrease the absorption of nutrition cholesterol and bile acids from the digestion track and affect the synthesis of lipoproteins. The content of UFA is desirable due to lower melting-point. Moreover those acids can positively affect the sensory quality, by reducing the specific "tallowy" taste. The adverse feature of the UFA is their tendency for rapid oxidation, which causes the rancidity of fat, lowering of the expiration date and creation of off-odors in fat with high content of C18:2, C18:3, C20:4, C20:5 and C22:6 (*Wood et al., 2004)*. CLA is an important

component of beef. CLA shows high antioxidant properties and inhibits the chemically induced skin, nipple, stomach and colon cancers. CLA is proved to have anti-diabetes activity, prevents from cell deterioration and stimulates the immune system. CLA similarly to stearic acid prevents the accumulation of cholesterol in human body. Beef meat contains on average 3,1-8,5 mg of CLA/g of fat, from which 73-93% is the c9, t11 isomer. This isomer is formed through hydrogenation of C18:2 and C18:3 by the enzymes secreted by *Butyrivibrio fibrisolvents* bacteria, present in rumen. The content of this isomer varies from 2,5-35 mg/100g of beef and is highly dependent on the animal diet. Meat of cattle fed naturally (grass), contains twice as much of this isomer then the meat of the cattle fed with commercial feeds. So the beneficial features of beef's fat are especially present in cattle grown on natural pastures. Beef is also a rich source of choline, used in the synthesis of lecithin (*Pisula et al., 2007)*. Choline takes part in transport and metabolism of cholesterol and other fats and influences the work of kidney and liver. Moreover beef contains high amounts of sphingomyelin and acetylcholine, which are involved in proper brain functioning. The content of cholesterol in meat varies from 40 – 85 mg/100g of meat, depending on the type of meat. The highest amounts of cholesterol are present in offal, from 100 – 380 mg/100g, depending on the animal and the type of offal. High quality beef meat contains very low amounts of cholesterol, when compared to other meat species (*Cierach, 2008)*. L-carnitine, another compound which is present in meat, plays an important role in metabolism and burning of fat in the cells. L-carnitine is formed in liver, and its synthesis requires vitamins (PP, C, B_6) and iron. Meat is the main source of L-carnitine.

High quality beef should be of intense red color, good tenderness and succulence, fresh scent, tight marbled structure and high nutrition value (*Dasiewicz et al., 1998*). Young beef is produced mostly from young bulls of dairy breeds or bidirectional utility breeds (for example Holstein-Fresian) or more frequently heifers and bulls from meat breeds. Consumers prefer the meat of red color and fine filamentary structure, tender, succulent, with appropriate taste and scent and easily digestible. Young beef should distinctively differ from veal acquired from the animals of the same race. Meats of all the species are neutral in flavor, since in meat the natural flavor/odor compounds are not present, just their precursors. Those compounds do not show the flavor/odor features of their own, but during the thermal treatment they react with other compounds forming new substances which give the meat positive flavor and scent. The high content of those precursors is essential, and one of the factors influencing it is the age of the animals. Cattle is usually subject to slaughter after achieving maturity and such meat contains high levels of precursors, which is why the mature beef is a meat of the most desirable sensory value. There is a reason why beef is a material for preparation of the best steaks like châteaubriand, filet mignon, porterhouse, t-bone steaks etc (*Grunert,*

1997). It is a meat free from parasites. On contrary to the pork meat it is not a processing meat but culinary meat, it is frequently consumed in its raw farm (steak tartar) or semi-raw (English steak). Tartar, or raw, chopped beef with addition of raw egg, onion or sometimes even pickled cucumber, is one of the flagship Polish dishes. In French kitchen, the steak tartar is minced in grinding machine and seasoned with Tabasco or Worcstershire sauces. Italian carpaccio is a very thinly sliced slices of raw beef, veal, salmon or tuna, topped with olive oil and lemon juice, served with lettuces and thinly sliced cheese. Coming from Chile Curdos, resembles the European tartar. Chopped beef is put on the slice of bread with addition of lemon juice, chopped onion and yoghurt or mayo based sauce. The national dish of habitants of Ethiopia and Eritrea is gored gored – raw beef cut in cubes, served usually with mild cheese or boiled green leafy vegetables or local bread. Turks and Armenians, on special occasions, prepare a dish called chee kufta, which is a raw beef or lamb served chilled as an appetizer. In many regions of Middle East, kibbeh nayyeh is a very popular dish. It is a chopped lamb, goat or beef served with various additives. The Koreans have their own version of tartar, called yukhoe, prepared from high quality beef meat, kidney, liver or heart. The animals from which such components are acquired have to be healthy and fed with special feeds during the fattening.

The diseases of second half of XX century turned out to be the prionic diseases, including BSE (mad cow disease), Scrapie in lambs, Chronic Wasting Disease (CWD) in deers and humans occuring: Crautzfeld-Jacob disease (CJD), kuru-kuru, Gerstmann–Sträussler–Scheinker syndrome (GSS) or fatal familial insomnia (FFI) *(Bosque, 2002)*. There are speculations that the new variant of Creutzfeld-Jacob disease (vCJD) in human can be caused be infectious prions, originating from diseased cattle suffering from BSE. It is suspected that the infection occurs through the digestive track as a result of consumption of meat products acquired from infected animals. Cheap hamburgers and other processed products – sausages, pies, minced roasts, stuffing of dumplings etc. could contain, next to meat, other components of beef carcass (brain, spinal cord, dorsal nerve roots). At present those parts of beef carcass are considered able to transfer the prions and are regarded as products of high virulence. Even the mechanically separated meat from the spine can be contaminated with neural tissue. Therefore there is an increased importance of histological test, especially the immunohistochemical tests of brain segments of dead and slaughtered animals. The initial cause of the prion diseases lies probably in the animal feeds. The rapid spread of the BSE was probably caused by feeding the cattle (ruminant animals, herbivorous) with powdered meat and bone feeds and it was correlated with more than one hundred cases of lethal Creutzfeld-Jacob disease in human who ate contaminated meat. In Great Britain, the whole herds of cattle from the disease

outbreaks were slaughtered and their bodies were burned – the overall of 4-5 million animals. In Poland all cases of BSE (76 until 2013) were discovered during the routine examinations of animals above 30 month old. The contaminated meat is destroyed and the remaining cattle form the farm is tested for the presence of the disease. The European Union does not close the borders for internal trade, because on all its territories (including Poland), the same security measures are performed. Ironically the outbreak of BSE contributed to increase in beef's quality, because the obligation to perform regular routine tests eliminates the meat of low quality.

Kulinarska vrednost goveđeg mesa

W. Migdał, B. Živković, J. Tkaczewska, P. Kulawik, A. Migdał, Ł. Migdał

Rezime

Kvalitet govedine i teletine sa stanovišta potrošnje odlikuje estetski izgled, ukus, mekoća i sočnost. Nutritivni kvalitet karakteriše sadržaj i sastav proteina, masti, vitamina, mineralnih komponenti i svarljivost. Tehnološki kvalitet je sposobnost vezivanja vode, pH, masti, proteina vezivnog tkiva i sadržaj i veličina komada mesa. Sanitarni kvalitet zavisi od količine mikroorganizama, rezidua štetnih materija i kontaminacije. Etički kvalitet zavisi od organske proizvodnje, verskih ubeđenja kao i socijalnih aspekata. Rast cena proizilazi i iz poboljšanog kvaliteta karakteristika govedine. Govedina pripada najvrednijim vrstama mesa u pogledu nutritivne vrednosti koja je određena prema približnom sastavu i sadržaju egzogenih jedinjenja.

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EFFECT OF METHANE FERMENTATION ON SURVIVAL OF ANTIBIOTICS-RESISTANT BACTERIA IN DAIRY COW MANURE

M. Iwasaki[1], M. Lukic[2], T. Yamashiro[1], M. Gogic[2], C. Radovic[2], F.J. Andriamanohiarisoamanana[1], K. Umetsu[1]

[1]Department of Animal and Food Hygiene, Obihiro University of Agriculture and Veterinary Medicine, Inada-cho, Obihiro, Hokkaido 080-8555, Japan
Corresponding author: umetsu@obihiro.ac.jp
[2]Institue for Animal Husbandry, Auto-put 16, Beograd, Zemun 11080, Serbia
Invited paper

Abstract: Utilization of biomass is essential for the development of a sustainable society. In particular, application of livestock manure, which is available in abundance, is not only ideal from the viewpoint of waste management, but is also valuable in terms of energy production. Consequently, many biogas plants are being built throughout the world, and these plants have the benefit of returning the digested manure byproduct of methane fermentation to farmlands in the form of liquid fertilizer. However, proper treatment and management of manure is necessary to prevent the spread of pathogenic and antibiotics-resistant organisms. We previously reported that methane fermentation reduces the number of pathogens. In this study, we found that antibiotics-resistant bacteria are present in the manure of dairy cow, regardless of administered antibiotics, and certain antibiotics-resistant bacterial strains remain in the manure after methane fermentation, albeit in small quantities. We plan to improve the efficacy of methane fermentation systems and develop a simpler and quicker method for detecting antibiotics-resistant strains.

Key words: methane fermentation, livestock manure, pathogens, antibiotics-resistant microbes, PCR–Denaturing gradient gel electrophoresis

Introduction

Methane fermentation is a livestock manure treatment method that exploits the decomposition of organic matter by anaerobic microorganisms. Under anaerobic conditions, organic matter is decomposed into methane and CO_2 by methane bacteria. Post-fermentation digested manure is used as liquid fertilizer owing to its high nitrogen, phosphate, and potassium content. In addition, digested manure is

easy to handle because of the breakdown of offensive-odor causing ammonia and volatile fatty acids. The rapid increase in biomass usage has prompted the urgent need for safety management standards for livestock waste and associated handling procedures. Furthermore, management and assessment of livestock manure handling are also needed to control communicable disease. Although we previously reported that methane fermentation reduces the number of pathogens *(Iwasaki et al., 2011)*, the potential spread of antibiotics-resistant bacteria via manure is a real concern because of the large amount of antibiotics used in livestock compared with humans. In addition to the treatment of infections, antibiotics are administered to dairy cow as a food additive to promote growth and improve feed conversion. In this study, we revealed the prevalence of antibiotics-resistant bacteria in livestock manure and investigated the effect of methane fermentation on them.

Materials and Methods

Cow manure

Raw cow manure and digested manure after methane fermentation were obtained from the biogas plant at dairy farm of Obihiro University of Agriculture and Veterinary Medicine. At this farm, cefazolin (SEZ), cefuroxime sodium (CFR), and penicillin (PC) are used to treat mastitis, PC and streptomycin (SM) to treat pneumonia and bronchitis, and neomycin (NEO) to treat bacterial gastroenteritis.

Detection of antibiotics-resistant bacteria

Raw manure and digested manure were smeared over an agar plate containing 50 μg/ml of an antibiotic agent, followed by incubation at 30 °C. Aerobic bacteria were cultured on LB medium plates under aerobic conditions and anaerobic bacteria were cultured on PTYG agar plates *(Fredrickson and Balkwill, 1998)* under anaerobic conditions. Colony-forming units were counted and compared.

Polymerase Chain Reaction–Denaturing Gradient Gel Electrophoresis

ISOFECAL for Beads Beating (NIPPON GENE, Tokyo, Japan) was used to isolate total DNA from raw or digested manure. Isolated DNA was used as a template in polymerase chain reaction (PCR) with specific primers *(Muyzer et al., 1993; González and Saiz-Jiménez, 2005)* against 16S rRNA, which is highly conserved among prokaryotes. PCR products were separated by (urea/formamide) denaturing gradient gel electrophoresis (DGGE).

Results and discussion

Antibiotics-resistant bacteria, including those resistant to antibiotics not in use at our farm, were detected in the manure of dairy cow, suggesting that the use of antibiotics did not significantly promote the development of antibiotics-resistant bacterial strains. Although antibiotics-resistant bacterial populations were reduced by methane fermentation, a higher number of bacteria resistant to antibiotics used

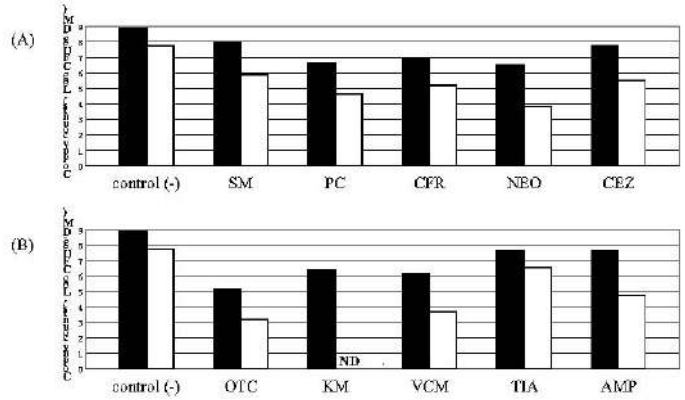

Figure 1. Detection of antibiotics-resistant bacteria
Antibiotics used (A) and not used (B) in dairy cow
■ manure; □ digested manure; ND, not detected

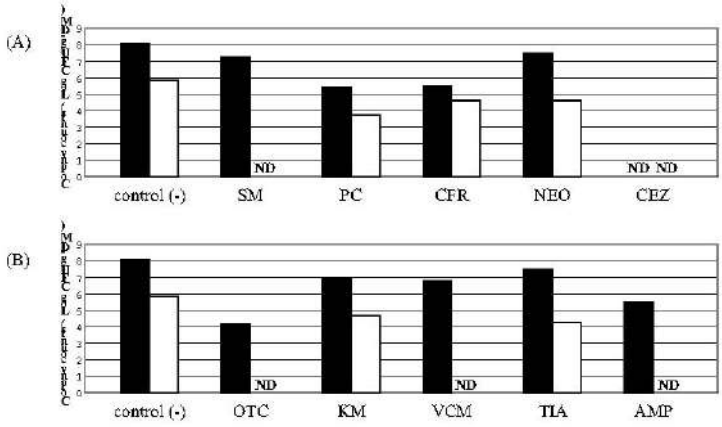

Figure 2. Detection of antibiotics-resistant archaea
Antibiotics used (A) and not used (B) in dairy cow
■ manure; □ digested manure; ND, not detected

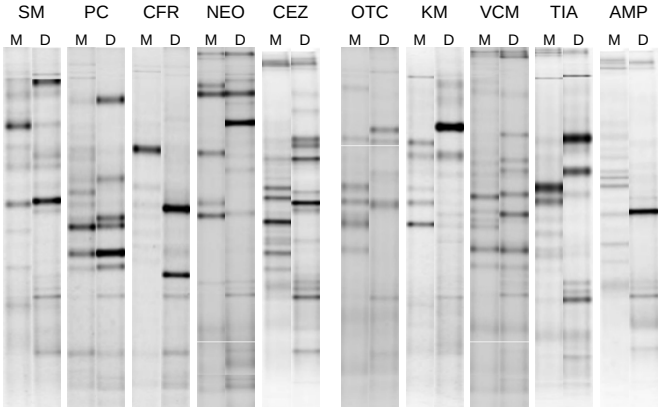

Figure 3. PCR-DGGE profiles of antibiotics-resistant bacteria
Samples: raw manure (M); digested manure after methane fermentation (D)
Liquid bacterial cultures were incubated for 2 days after the addition of each antibiotic agent.
Antibiotics used (SM, PC, CFR, NEO, and CEZ) and not used (OTC, KM, VCM, TIA, and AMP) at Obihiro University of Agriculture and Veterinary Medicine.

at our farm remained after fermentation compared with bacteria resistant to antibiotics not in use at our farm (Figure 1, A, B).

Although archaea resistant to certain antibiotics disappeared after methane fermentation, a fermentation-induced reduction in the number of archaea did not differ significantly depending on the antibiotics used (Figure 2, A, B).

PCR-DGGE was used to investigate antibiotics-resistant bacteria in manure samples before and after methane fermentation, and the results showed the presence of various strains (Figure 3).

A comparison of raw and digested manure after electrophoresis showed that only a few common bands between the two samples treated with SM, CFR, CEZ, KM, TIA, and AMP. This suggests that the original antibiotics-resistant microbes were killed by methane fermentation and were subsequently replaced by other antibiotics-resistant strains. On the other hand, PC and VCM-resistant microbes in raw manure appear to have survived methane fermentation.

Antibiotic resistance can be acquired through (1) gene mutation involving one to several base pairs, (2) the transmission of a plasmid containing a antibiotics-resistant gene, and (3) recombination of transposons. In addition, the structure of an antibiotic agent may promote cross resistance and co-resistance. Regardless of the cause, any microbial strains, not limited to specific ones, can acquire antibiotic resistance. In addition to continue identifying antibiotics-resistant microbes in the future, we plan to elucidate the prevalence of antibiotics-resistant microbial strains after methane fermentation at an ultra-high temperature (>65 °C) because a higher

number of antibiotics-resistant microbes are reduced at higher temperatures during methane fermentation *(Iwasaki et al., 2011)*.

PCR-DGGE followed by sequencing analysis is the most reliable way to determine microbial strains including related, but currently unknown strains. However, the technique is costly and time consuming and is thus not suitable for screening. On the other hand, PCR detection of specific DNA sequences can be an effective screening tool because it is easy to prepare and generates results quickly. Therefore, to apply PCR for the quick identification of antibiotics-resistant microbial strains, including unknown strains resistant to a specific antibiotic, it is necessary to develop a technique to simultaneously identify the sequence of the antibiotics-resistant gene and the sequence specific to the microbial strains.

Conclusion

The effective application of biomass resources including livestock manure is a prerequisite for the establishment of a sustainable society. However, because of the presence of antibiotics-resistant microbial strains in livestock manure, proper treatment of manure is needed to ensure its safety as a resource. Methane fermentation can not only generate energy and good fertilizer, but also reduce antibiotics-resistant microbes in manure and thus function as a sanitary management system. However, because certain antibiotics-resistant microbial strains can survive methane fermentation, further studies are needed to investigate high and ultra-high temperature methane fermentation or electrochemical reactions and to develop safe and sustainable technology for the recycling of resources. In addition, a simpler and quicker PCR method without culture methods is needed for the monitoring of pathogenic and antibiotics-resistant microbes.

Uticaj fermentacije metana na preživljavanje bakterija rezistentnih na antibiotike u stajskom đubrivu mlečnih krava

M. Iwasaki, M. Lukić, T. Yamashiro, M. Gogić, Č. Radović, F.J. Andriamanohiarisoamanana, K. Umetsu

Rezime

Korišćenje biomase od suštinskog je značaja za razvoj održivog društva. Posebno, primena stajnjaka koji je dostupan u izobilju, koji je idealan ne samo sa stanovišta upravljanja otpadom, već je važan i u smislu proizvodnje energije.

Shodno tome, mnoga biogas postrojenja se grade širom sveta, a korist od ovih postrojenja je u vraćanju proizvoda od fermentacije metana koji nastaje digestijom sporednih proizvoda stajnjaka na farmi u obliku tečnog đubriva. Međutim, pravilno postupanje i upravljanje đubrivom je neophodno da bi se sprečilo širenje patogena i organizama otpornih na antibiotike. Mi smo ranije objavili da fermentacija metana smanjuje broj patogena. U ovoj studiji smo otkrili da bakterije otporne na antibiotike su prisutne u stajnjaku od mlečnih krava, nezavisno od propisanih antibiotika, a pojedine bakterije otporne na antibiotike ostaju u đubrivu i posle fermentacije metana u malim količinama. Planiramo da poboljšamo efikasnost sistema fermentacije metana i razvijemo jednostavniji i brži način za otkrivanje antibiotik-rezistentnih sojeva.

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NEOSPORA CANINUM IN CATTLE: EPIZOOTIOLOGY, DIAGNOSTICS AND CONTROL MEASURES

B. Vidić[1], S. Savić[1], S. Boboš[2], V.Vidić[1], N. Prica[1]

[1]Scientific Veterinary Institute „Novi Sad“, Rumenački put 20, 21000 Novi Sad
[2]Department of Veterinary Medicine, Faculty of Agriculture Novi Sad, Trg Dositeja Obradovića 8, 21000 Novi Sad
Corresponding author:branka@niv.ns.ac.rs
Invited paper

Abstract: *Neospora caninum* is a coccidian parasite and well established pathogen for cattle, which is responsible for numerous abortions. The organism is worldwide distributed in dairy and fattening cattle. Neosporosis can occur at *sporadic***,** *endemic,* and *epidemic* levels in herds. Dog and other carnivores are the primary hosts for *N. caninum*, and the infection is disseminated via feces of infected animals excreting oocysts in the environment. Cattle get infected by ingesting the oocysts in feed and drinking water. Transplancental route (vertical transmission) is the most common transmission route. The agent is transmitted to the developing embryo or fetus, and severity and outcome of such infections are determined by the pregnancy stage. The abortions occur mainly between the 3rd and 7th month of pregnancy. Besides dogs, birds and poultry are reported as potential source of infection.The percentage of seropositive cows in the world population of dairy cattle ranges from 5-65%. In some areas, it was established that *N. caninum* is responsible for more than 30% of all abortions in cattle. Little is known about the risk factors for *N. caninum* infection; however, contact between cattle and dogs is considered highly hazardous. Some research revealed highly positive correlation between seropositivity in dogs and prevalence of *N. caninum* infection in cows. Laboratory diagnosis is established by agent detection by the method of immunohistochemical staining (IHC), immunofluorescence antibody test (IFAT), Western blot method (WB) and PCR or by detection of specific *N. caninum* antibodies applying direct agglutination test (DAT), ELISA and cELISA. ELISA test proved adequately specific and sensitive to identify the infection at herd level.
This article gives and overview of importance and role of *N. caninum* in the pathology of dairy cattle diseases. Our aim was to outline contemporary knowledge and potential risk factors at dairy farms in Serbia, as well as the available control measures. This paper offers the analysis and comparison of pathogen prevalence in dairy herds in Serbia as well as its role in the abortions.

Key words: *Neospora caninum*, cows, epizootiology, diagnostics, control measures

Introduction

Neospora caninum (*Apicomplexa*) is a coccidian parasite and well established causative agent of the disease, primarily in dogs and cattle worldwide. The parasite was first recognized in Norway in 1984 in dogs manifesting symptoms of myositis and encephalitis. The organism is described and named *Neospora caninum (Bjerkas et.al., 1984)*. In 1988, *N. caninum* was recognized as a causative agent for abortions in cows *(Dubey et al., 1988)*, and has emerged as an important pathogen causing reproductive disorders and abortions in numerous countries *(Haddad et al., 2005; Vidić et al., 2011c)*. In cattle, the *N. caninum* infection often can take a subclinical course usually resulting in reduced milk yield, reduced growth in fattening herds and poor food conversion. Thus, considerable economic losses are likely in dairy cows and beef cattle. Records on prevalence of cattle neosporosis in numerous European countries strongly indicate high percentage of seroreactor animals in dairy herds *(Pare and Hietala, 1995; Davison et al., 2001; Otranto et al., 2003; Schares et al., 2003)*. A recent research in Sweden and Holland revealed the rates of seropositive cows being 16% and 76%, respectively *(Bartels et al., 2006)*.

Clinical neosporosis was identified in sheep, goats, deers, rhinoceroses, horses and experimental animals. Antibodies against *N. caninum* were detected in buffalos, foxes, coyotes and camels, indicating that wild animals are potential infection reservoirs *(Godmin et al., 2004).*

Etiology and epizootiology

Dogs proved to be definitive hosts for *N. caninum.* Dogs shed the unsporulated cysts in the feces, and sporulation occurs outside the host, in the environment *(Vidić et al., 2011a)*. Dogs can excrete infectious oocysts during undefined time after infection onset, and data on the survival of oocysts in the environment are still lacking *(Lindsay and Dubey, 2000)*. In bitches, the infection is mostly subclinical and transplacental transmission of the parasite results in bringing forth the infected offspring *(Bjerkas et al., 1984)*. The most commonly observed symptom in these animals involves hind limb paresis, which progresses to paralysis. Such dogs can survive even several months. In dogs older than six months, muscular and neurological symptoms are dominant, including head tilt, swallowing difficulties, jaw paralysis, muscular pain and muscle atrophy.

Moreover, dyspnoea, pneumonia, hepatitis, ulcerous dermatitis and sometimes encephalitis can be observed *(Peters et al., 2000; Cantile and Arispici, 2002)*. The disease occurs as localized or generalized form that may involve all organs, including skin.

Since the disease was first registered, *N. caninum* antibodies were detected in 37.8% dogs in Argentina, 22% in New Zealand, 10% in Turkey, 6.7% in Brazil and in 6.4% dogs in Italy *(Lindsay and Dubey, 2000)*. Neosporosis affects dogs of all age categories.

The life cycle of *N. caninum* is typified by three stages: tachyzoites, tissue cysts, and oocysts. Tachyzoites and tissue cysts are the stages observed in the intermediate hosts, and they are localized intracellularly *(Haddad et al., 2005)*. Carnivores become infected by ingesting infected tissues containing tissue cysts with bradyzoites. Vertical (transplacental) infection is the dominant transmission route in cattle; however, horizontal infection route via feed and water contaminated with sporulated oocysts of *N. caninum* is possible *(Davison et .al., 2001)*. Neosporosis has been diagnosed in other animal species *(Vidić et al., 2008)*. In sheep, *N. caninum* was first identified in England, in a congenitally infected lamb. Abortions associated with suspect *N. caninum* infection were reported also in dairy goats *(Haddad et al., 2005)*.

Bovine neosporosis

Bovine infections associated with coccidian parasite *N. caninum* have been reported in almost all countries worldwide: Australia, New Zealand, European countries, South Africa, Korea, Japan, Thailand and America *(Moore et .al., 2003*; *Haddad et al.,* 2005; *Vidić et al., 2011b)*. Quantitative studies carried out in particular geographic areas identified *N. caninum* as a major cause of abortions and neonatal deaths in dairy and beef cattle, showing the highest prevalence in the U.S.A., New Zealand, Netherlands and Germany *(Rodrigez et al., 2002)*. Serological prevalence in cattle varies according to country region *(Pare and Hietala, 1995; Packham et al., 1998)*, applied serology test and number of investigated animals and ranges between 87 and 90% *(Davison et al., 2001; Dubey, 2003)*. Some 95% congenitally infected calves originating from seropositive cows are born clinically normal, healthy, although chronically infected *(Moore et al., 2003; Haddad et al., 2005)*. The age of the cow, number of lactation cycles and abortion history do not affect the rate of congenital infection of the embryos in a subsequent pregnancy *(March et al., 1998; Moore et al., 2003)*. *Neospora* abortions may occur from three months of gestation to term; however, abortions associated with *N. caninum* infection typically occur at mid gestation, i.e. between 5th and 6th gestation month. In addition to abortion, fetal deaths in utero as well as

resorption, mummification or autolysis of the fetus have been associated with *Neospora* outbreak. The newborn calves can manifest clinical signs of abnormalities but majority of them have no clinical signs of the disease yet being latent carriers. Seropositive cows (carriers of *N. caninum* antibodies) are more prone to abortions than the seronegative ones. The period from 4th to 5th month before calving is characterized by increase in antibody level, suggesting the reactivation of latent infection. Parasitemia that occurs in this period of gestation results in the infection of the fetus *(Buxton et al., 2002)*.

To date, there is no evidence on direct transmission of *N. caninum* between cows; the transplacental infection is the most common infection route. So far, there is no evidence on either genital transmission or infection via the embryo transfer. Thus, embryo transfer is even recommended as the effective measure for the control and prevention of vertical transmission of the agent. Transmission of *N. caninum* via the milk has been experimentally confirmed; however, there is no evidence that lactogenic transmission of *N. caninum* occurs in nature. Dogs fed milk inoculated with *N. caninum* tachyzoites did not excrete oocysts in the feces. Clinical symptoms of bovine neosporosis were so far reported in animals younger than two months. Infected calves demonstrated decreased body mass, inability to rise or neurological symptoms such as ataxia, poor patellar reflex, significant loss of response to environmental stimuli, exophthalamus, hydrocephalus and spinal channel restriction. Abortions may occur as endemic or epidemic ones. Abortions occurring in 10% or more of cattle population within the time period of 6-8 weeks are considered epidemic *(Haddad et al., 2005)*.

Infection is thought to reduce milk yield in dairy cows through its effects on fertility as cows that have aborted often have a lower milk yield and are more likely to suffer retained fetal membranes. *Neospora* infections in young calves have been reported to cause neuromuscular disease and may affect growth rate of finishing animals *(Innes et al., 2002)*. Direct and indirect economic losses associated with this coccidium implicate the price of the lost calf, expenses of the expertise and diagnostics, costs of individual animals until returns to service, decreased milk production as well as costs for the replacement of culled animals.

Diagnosis

Bovine neosporosis is manifested by early embryonic death, abortion, stillborn or non-vital calf as well as by delivering normal calf without apparent clinical manifestations *(Innes et al., 2002)*. Clinical manifestations in cows infected with *N. caninum* are associated with two major factors, i.e. whether the animal is pregnant at the moment of infection and at which gestation stadium (early, mid or late gestation period). Non-pregnant cows typically do not manifest any clinical

symptoms. Seroconversion, i.e. presence of specific *N. caninum* antibodies in blood or milk serum is the only indicator of the infection.

If the dam gets infected at the beginning of gestation period (2-3 months), early embryonic death is likely to occur. In *Neospora* infections acquired from 3rd to 7th gestation month, abortions, delivery of non-vital calves or calves with diverse disorders may occur, depending on the gestation month *(Buxton et al., 2002)*. In infections acquired by the end of gestation period, weakly vital or normal calves without symptoms yet seropositive to *N. caninum* are delivered. During this gestation stadium, the immune system of the calf better responds to infection, which results in only limited or not at all consequences for the newborn animal *(Innes et al., 2001)*.

Since the first identification of N. caninum, a range of diagnostic tests have been developed and assessed. The most widely used methods include immunohistochemical staining, indirect immunofluorescence, ELISA, direct agglutination, Western blot analysis and PCR *(Lindsay and Dubey, 1989; Pare and Hietala 1995; Packham et al., 1998; Romand et al., 1998)*. The only indicative diagnostic procedure for identification of *N. caninum* is examination of the serum of aborted cows. Confirmation of the diagnosis of neosporosis requires histopathological examination of aborted fetuses. Most commonly, samples of the brain, heart, liver, placenta and tissue liquids are submitted for examination. If possible, blood serum should be examined, too. Although characteristic changes are observed in multiple organs of the fetus, major changes are most clearly seen in the brain. Since most aborted fetuses are autolyzed, partly digested brain samples should be submitted for histopathology. Mostly small amount of *Neospora* organisms is present in fetal brain, thus immunohistochemical examination of tissue portions is required. Histopathological changes mostly implicate focal encephalomyelitis (associated with necrosis and non-suppurative inflammation), hepatitis (occurring more often in epizootic abortions than in sporadic ones) and placental changes. It is to be emphasized that detection of this coccidium in the placenta is quite complex and intricate process.

Although immunohistochemical identification of *N. caninum* offers the best evidence in the etiology of abortion, this method is nowadays considered highly non-specific. Another diagnostic possibility is PCR detection of *N. caninum* DNA in brain preparations of the aborted calf. The efficacy of PCR in the diagnostics of this disease is closely associated with the laboratory itself, sampling procedure and degree of fetal autolysis *(Lally at al., 1996)*.

Detection of *N. caninum* antibodies by serodiagnosis includes specifically modified ELISA (enables differentiation between acute and chronic infections), indirect fluorescent antibody (IFA) test, direct agglutination test (enables detection of IgG class) and immunoblotting assay *(Harkins et al., 1998; Sondgen et al., 2001*;

Sager et .al., 2003). Although a valid serological diagnosis of neosporosis can be established using the blood serum or any fetal body fluid, sampling of fetal peritoneal fluid is highly recommended. For a serodiagnosis of congenital infection, calf serum should be collected immediately before obtaining of colostrum. Detection of *N. caninum* antibodies in calves' sera indicates the existence of this coccidial infection. Negative result does not necessarily exclude the infection considering that synthesis of fetal antibodies is strongly dependent on gestation stage, exposure level and the time period between infection onset and abortion. Antibody titer of 1:25 can be considered specific for *N. caninum,* especially in the fetus.

Besides *N. caninum*, *T. gondii* is another potential causative agent of bovine abortions of protozoan etiology. Because of morphological similarities of *N. caninum* and *T. gondii* etiological diagnosis should be obtained by an immunohistochemical examination and PCR detection of the parasitic DNA. The attempts to isolate active forms *of N. caninum* have mostly failed and antigenic properties of diverse *N. caninum* isolates are still unclear, especially when speaking of healthy animals. Isolation of *N. caninum* form the fetus was unsuccessful, since the pathogen population dies inside the host. This coccidium can be more readily isolated from neural tissue of congenitally infected calves, as neural tissue contains the tissue cysts, which are much more resistant to autolysis than the *N. caninum tachyzoites (Lindsay and Dubey, 2000; Dubey, 2003).*

Control measures

Neosporosis has proved to be highly challenging disease in a view of treatment and control *(Kljajić and Vidić, 2010)*. However, some antimicrobials applied in the therapy of toxoplasmosis (sulfadiazine, clindamycin) proved applicable. Potential prevention measures include timely detection of seroreactor animals, separation of the offspring from infected mothers, disease monitoring in low-prevalence herds, prevent dogs to access aborted fetuses, placenta or meat of dead cattle as well as prevent fecal contamination of feed and drinking water in the herd *(Haddad et al., 2005)*. So far, reliable and effective vaccine that might prevent abortions is not available. Inactivated vaccine was applied in experimentally infected cattle, resulting in prevention of transplacental transmission of the parasite *(Innes et al., 2002)*. In this way, effective protection from abortion was provided only in case that immunity had been developed prior to the exposure to primary infection.

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Neospora caninum kod goveda: epizootiologija, dijagnostika i mere kontrole

Branka Vidić, Sara Savić, S. Boboš, V.Vidić, N. Prica

Rezime

Neospora caninum je protozoa, kokcidija, poznata kao uzročnik pobačaja kod krava. Rasprostranjen je širom sveta kod mlečnih i tovnih grla. Neosporoza se javlja sporadično, endemski i epidemijski kod krava. Domaćin za *N.caninum* je pas i drugi karnivori, koji šire infekciju preko fecesa kada luči oociste u spoljašnju sredinu. Goveda se inficiraju ingestijom oocista hranom i vodom. Uzročnik se transplacentarno, vertikalna transmisija, prenosi na plod i posledice takve infekcije su različite i zavise od faze graviditeta. Abortusi najčešće nastaju između 3-7 meseca graviditeta. Pored pasa rezervoari infekcije mogu biti i ptice i živina.
Procenat seropozitivnih krava u svetu kreće se od 5-65% kod mlečnih krava. U nekim područjima utvrđeno je da je *N.canunum* odgovorna za više od 30% svih pobačaja kod krava. Faktori rizika za infekciju nisu još dovoljno proučeni, ali kontakt krava i pasa se smatra visoko rizičnim. Ispitivanja koja su vršena pokazala su da je seropozitivnost pasa sa farmi u visokoj korelaciji sa prevalencijom infekcije kod krava. Laboratorijska dijagnostika vrši se direktnim dokazivanjem uzročnika imunohistohemijskim bojenjem (IHC), metodom imunofluorescencije (IFAT), Western blot metodom (WB) i PCR, ili dokazivanjem specifičnih antitela za *N.caninum* metodom aglutinaicje (DA), ELISA i cELISA. ELISA test se pokazao dovoljno specifičan i osetljiv za ispitivanje prisustva infekcije na nivou zapata.

U radu su prikazana saznanja o značaju i ulozi *N.caninum* u patologiji muznih krava. Cilj rada je da se prikaže pregled sadašnjih saznanja i potencijalne faktore rizika za farme krava u Srbiji i mere kontrole koje su na raspolaganju. U radu će biti analiziran i upoređen stepen prevalencije patogena u zapatima mlečnih krava u Srbiji i uloga patogena u pobačajima.

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DISPERSION PARAMETERS FOR LITTER SIZE IN PIGS

Z. Luković[1], D. Radojković[2]

[1]University of Zagreb, Faculty of Agriculture, Department of Animal Science and Technology, Svetošimunska 25, 10000 Zagreb, Croatia
[2]University of Belgrade, Faculty of Agriculture, Institute of Zootechnics, Nemanjina 6, 11080 Belgrade-Zemun, Serbia
Corresponding author: lukovic@agr.hr
Invited paper

Abstract: Litter size is one of the most economically important traits and number of piglets born alive is the selection trait of choice in improvement of litter size in most pig breeding programmes. Litter size is affected by numerous environmental and genetic factors and interactions between them. The use of the mixed model methodology has become a standard to estimate dispersion parameters by residual maximum likelihood method (REML) and to predict breeding values of animals by best linear unbiased prediction (BLUP). There are several approaches regarding the method for genetic evaluation of litter size. They mainly depend on genetic correlations between litter size in subsequent parities, size of data set, capacity of computing, and also data quality. Due to simplicity the genetic evaluation of litter size in many countries based on repeatability model. Low genetic correlations between litter sizes in different parities are reason for use of multiple trait analysis, although it is rarely used. In the recent period, random regression models were applied to estimate dispersion parameters for litter size. Although many studies about dispersion parameters for litter size include large scale farms and modern pig breeds, increase of population size of some local pig breeds opens a possibility to renew breeding programme for these breeds and to determine dispersion parameters for litter size as a trait usually recorded for management purpose.

Key words: pigs, litter size, dispersion parameters

Introduction

From statistical point of view, effects can be classified as fixed or random *(Searle et al., 1992)*. The effects are considered as fixed whenever they can be attributed to a finite set of levels in the data and which are there because we are interested in them. For fixed effects, there must be usually enough information in the data. Many environmental and genetic factors, as well as complex interactions

between them, influence litter size in pigs. They could be arranged into two major groups *(Clark and Leman, 1986a)*. The first group includes factors which are often recorded by commercial pig producers and contains effects like parity, sow breed or genotype, age at farrowing, lactation length, weaning to conception interval etc. The data were used for management and selection purposes. The second group includes factors such as husbandry practices, nutrition, and diseases. Although they are very important, data on these factors are not always available and often cannot be evaluated.

Random effects are attributable to a (usually) infinite set of levels, of which only a random sample is considered to be included in the data. Additionally, random effects usually have a small number of observations per level and at the same time a large number of levels. Random effects could be genetic or environmental. In most animal breeding applications, only additive genetic effects are considered in the evaluation of animals. Beside additive genetic models, there is an increased interest in models that consider non additive genetic effects, as dominance. The sows influence on the performance of her offspring is not limited only to genetic effects, but also includes environmental random effects as permanent and common litter effects. The knowledge of variances and covariances of random effects that affect litter size is necessary to define how breeding values should be estimated.

Random effects in the models for litter size

Additive genetic effect is a sum of mostly small allele effects of the many genes. A half of the alleles carried by each parent are passed from parents on their progeny. The measure of animal genetic effect is the additive genetic effect and its proportion (heritability) in total phenotypic variance. Estimates of heritability vary mainly from 0.0 to 0.2. The general conclusion is that the heritability of litter size is around 0.10, as reviewed by *Rothschild and Bidanel (1998)*. Although estimates of heritability for litter size are generally low, direct additive genetic variance was sufficient to obtain significant genetic progress *(Southwood and Kennedy, 1990; Chen et al., 2003)*. Heritability estimates are slightly but not significantly higher for the number of piglets born total than for the number of piglets born alive *(Mercer and Crump, 1990; Roehe and Kennedy, 1995; Hanenberg et al., 2001)*. Evidently there is a variation in heritability for litter size between parities. Lower heritabilities were estimated in the first parity compared to the last parities *(Roehe and Kennedy, 1995)*. *Alfonso et al. (1997)* found high heterogeneity in heritability estimates (from 0.01 to 0.09) using univariate analyses for the first five parities in Landrace populations. Heritabilities in the first four parities obtained by multivariate analyses were lower than the ones from univariate analyses and ranged

from 0.02 to 0.04. Heritability estimates for liveborn piglets in the first six parities using the combination of two trait analysis varied mainly between 0.05 and 0.25 in the study of *Kovac and Sadek-Pucnik (1997)*. Exceptionally high estimates were found in two trait analyses for higher parities and *Kovac and Sadek-Pucnik (1997)* explained them as a consequence of numerical problems. Heritabilities were lower in the multiple trait than in the univariate analyses *(Hanenberg et al., 2001)*. Lower heritabilities for NBA were often found in the analyzes of local pig breeds (*Perez-Enciso and Gianola,1992; Lukovic et al., 2010)*, but sometimes also in the modern breeds (*Radojkovic et al., 2012)*. Breed differences in heritability may exist *(Gu et al., 1989; Babot et al., 1994)*, but there is little reliable evidence for them *(See et al., 1993; Chen et al., 2003)*. *Irgang et al. (1994)* reported that higher estimates of heritability for some breeds indicate the opportunities for genetic improvement of litter size in these breeds that may be greater than in others. In the study from *Ferraz and Johnson (1993)*, two of four estimates of heritability for litter size in Yorkshire and Landrace sows, in two herds by breed, were zero. Authors explained so low estimate of heritabilities as a consequence of small data sets. *Kisner et al. (1996)* reported estimates of heritabilities in the first three parities in a wide range from zero to 0.10 and explained them also as a result of small number of records in the data set, as well as data structure. Several reasons for the heterogeneity of heritability estimates were reported by *Southwood and Kennedy (1990)*. The main reasons were random estimation error, breed and time at which litter size is measured, unaccounted genetic and environmental sources. *Southwood and Kennedy (1990)* also found that heritability estimates were reduced considerably by not accounting for maternal effect and its correlation with direct additive genetic effect, but only for one of two breeds in the study. However, the results of their study were not consistent with the findings of *Crump et al. (1997)* who observed small changes when maternal genetic effect was included in the model. Estimates of heritabilities are a function of variance components and are, in general, specific for a particular population and period of time *(Kaplon et al., 1991)*.

Maternal effect indicates that dam has an influence on the performance of her offspring. The maternal effect of a sow is a function of both her genotype and environment. Intrauterine environment, milk production, and mothering ability of the dam may affect her offspring's reproductive performance. Although maternal effects are strictly environmental with respect to offspring, these effects can have both environmental and genetic components. In practice, the maternal environmental effect would be accounted for by the common environmental effect of birth of a sow *(Roehe and Kennedy, 1993b)*. The presence of maternal additive genetic effect may bias estimates of direct additive genetic effects because both are transmitted from one generation to the next. Genetic differences among dams are expressed as phenotypic differences of their offspring when they become dams,

i.e., the additive maternal effect is expressed one generation later than the additive direct effect. Estimates of maternal genetic effect were low and ranged from 0.00 to 0.05 *(Chen et al., 2003)*. Genetic progress for litter size may be reduced because of negative correlation between direct additive genetic and maternal additive genetic effects. It has been suggested that an antagonistic genetic correlation between direct and maternal genetic effects may be responsible for the observed low heritability and lack of response to selection for litter size *(Southwood and Kennedy, 1990)*. Simulation study by *Southwood and Kennedy (1990)* showed that maternal additive genetic effect with negative correlation to direct additive effect from the first parity litters had a considerable effect on response to selection of litter size. The importance of maternal additive genetic effect has been controversial. Using REML under an animal model, *Mercer and Crump (1990)* and *Perez-Enciso and Gianola (1992)* found no maternal effect, whereas *Southwood and Kennedy (1990)* and *Ferraz and Johnson (1993)* reported significant maternal effect. *Ferraz and Johnson (1993)* compared models without and with maternal additive genetic effect and tested them using likelihood ratio test. Low maternal heritability could be explained by the large amount of crossfostering practice, often within 24 hours from birth, which means that sows who were litter mates at birth did not necessarily share the same postnatal environment *(Crump et al., 1997)*. A negative genetic correlation between the direct and maternal effect could lead to conflict in the improvement of a trait. Although the maternal environmental component may be removed by appropriate management or statistical methods, such as crossfostering or adjustment of the data, the genetic part may not be accounted for. *Roehe and Kennedy (1993a)* noticed that maternal genetic effect can have a high influence on genetic improvement of litter size, even when maternal heritability is low compared to direct heritability, depending on the genetic correlation between maternal and direct genetic effects. Various reasons for contradictory results for maternal effect are possible. Different populations, different environmental conditions, crossfostering etc., are the most frequent reasons listed. Different models and traits used could also have an influence on the estimates of the maternal effects. *Vangen (1981)* showed that maternal effect mainly affects the first litter. Therefore, maternal effect, if important in the population, should be included in the evaluation model for the first parity litter *(Roehe and Kennedy, 1993b)*. In subsequent parities, the statistical model may be different because of the less important maternal effect.

Sire influences litter size directly and indirectly. Service sire as a father of the litter affects litter size directly through semen characteristics (quality and quantity) and through genetic potential for development and survival of embryos. Sire, as a father of the sow, affects litter size indirectly through fertility of his daughters which is described by direct additive genetic effect. It is necessary to

distinguish service sire of the litter and sire of the sow. Although sire effect presents mainly a genetic effect, it can also include environmental components. Different semen dilution as well as different interactions between animal and human at mating time can be the reason for differences in litter size. With a reported estimate up to 5 % of the total variation in the number of piglets born alive, it seems that the service sire has a small but significant effect on the number of piglets born alive *(See et al., 1993; Hamann et al., 2004)*. Service sires within genotype could produce substantially larger or smaller litters in relation to average litter size in a population. Sires can produce small litters if semen concentration and quality are so low that not all eggs are fertilized *(Clark and Leman, 1986b)*. Individual sires can produce small litters if lethal genes are produced that result in death of a portion of embryos *(Ollivier, 1982)*. Litter size is then typically reduced by 50 to 75 % and more due to an increased embryonic mortality. Estimates of the contribution of the sire of a litter to the variance in litter size are generally small, and seem dependent on whether service is natural or by artificial insemination *(Strang, 1970; Haley et al., 1988)*. *Southwood and Kennedy (1990)* assumed that service sire did not have an important role on litter size. The effect, which may be seen as 'fetal effect' on sow prolificacy, explains up to 5 % of the total variance in litter size *(Ollivier, 1982)*. However, *Ollivier (1982)* argued that service sire effect is more important than those explained by the genetic effect transmitted by the sire to his daughters. Therefore, a possibility exists, to maintain litter size at a satisfactory level by culling the less prolific sires.

Common litter environmental effect describes common environment shared by sows from farrowing to weaning, and in some cases, growth period if animals continue to stay together after weaning. Because of common environment, resemblance between litter mates as well as diversity among different litters increase. Variance of common environment is caused by microclimate, milk production, other maternal ability, nutrition, hygiene and so on. Although the effect presents the usual environmental component, common litter effect contains some genetic components like dominance and maternal genetic effects, if they are not included in the model *(Bolet et al., 2001)*. Estimates of common litter environmental effect were low and ranged usually between 0 and 6 %. *Crump et al. (1997)* considered that lower estimates of the effect may be a consequence of the routine practice of crossfostering. *Kaplon et al. (1991)* found higher estimates in range between 0.03 and 0.11. *Kaufmann et al. (2000)* reported that common litter environmental effect explains 6 % of phenotypic variance. Small magnitude is often described as a consequence of a small number of litter mates in the data. If more than 70% of the records presented sows with no litter mate or the average number of sows per litter was below 1.5, *Southwood and Kennedy (1990)* suggested that the effect could be assumed to be unimportant and thus, not included

in the model. *Hermesch et al. (2000)* agreed that data containing less than 20 % of sows with one full sister should be analysed to fit litter effect as an additional random effect. Adding common litter effect to the animal model did not cause any sizable reduction in the residual variances or change of heritabilities *(Irgang et al., 1994)*.

Permanent environmental effect is connected with more measurements on the same animal. Because of the same permanent environment, measurements obtained on the same animal are more similar than measurements obtained on the others. Frequently, estimates ranged between 5 and 10 %. *Alfonso et al. (1997)* and *Hanenberg et al. (2001)* estimated that 7 to 9 % of phenotypic variance was explained by permanent environmental effect. In both cases random part comprised only direct additive genetic and permanent environmental effects. *Chen et al. (2003)* considered additional service sire and maternal additive genetic effect in the model and obtained estimates for permanent environmental effect between 0.06 and 0.08 for four different breeds. The importance of including this effect in the model was given by *Ferraz and Johnson (1993)*, which reported estimates of permanent environmental effect as a ratio in phenotypic variance in the range between 0.16 and 0.17. On the other hand, *Adamec and Johnson (1997)* found lower estimates of permanent environmental effect, in the range between 0.02 and 0.03.

Methods for estimation of dispersion parameters

There are several approaches regarding the method for genetic evaluation of litter size. They mainly depend on genetic correlations between litter size in subsequent parities, size of data set, capacity of computing etc. Due to simplicity many pig breeding organizations use repeatability model for the estimation of breeding values for litter size *(e.g., Alfonso et al., 1997; Noguera et al., 1998*). A repeatability model assumes that genetic correlations between litter size in subsequent parities are equal to one and that the variance is constant along this trajectory. On the other hand, multiple trait analysis supposes subsequent observations to be different traits. Estimates of genetic correlations between litter sizes in different parities are often lower than one *(Alfonso et al., 1994; Irgang et al., 1994)*, especially between the first and later parities *(Serenius et al., 2003)*. The low correlations between parities indicate that partly different genes are responsible for litter size in different parities. Therefore, multiple trait analysis is preferred in such situation in order to increase the efficiency of selection. Beside a simple repeatability model and a full multiple trait model, a possible approach of handling repeated observations would be to use covariance functions and random regression model. Litter size in pigs is a repeated measure and could also be considered as a

longitudinal trait *(Schaeffer and Dekkers, 1994)*. Main advantages of the random regression approach in comparison to the multiple trait approach are: smaller number of parameters to describe the longitudinal process, smoother covariance estimates, as well as a possibility to estimate covariance components and predict breeding values at any point along the trajectory. Although litter size differs from typical longitudinal traits such as growth random regression could be also applied for the genetic evaluations *(Schaeffer, 2004; Lukovic et al., 2004; Lukovic et al., 2007)*.

Conclusions

Knowledge of genetic and environmental parameters for litter size in pigs is necessary to estimate accurate breeding values, to optimize breeding schemes, and to predict response to selection. For estimation of genetic parameters of litter size in pigs different methods can be used. With the assumption of unit genetic correlations between parities and constant variance along parities the repeatability model is the model of choice. In the situation when genetic correlations between parities are lower, the multiple trait model is suggested. Random regression model can be used instead of the multiple trait model in such cases to lower the number of parameters to estimate. However, sufficient data with proper structure is needed to apply random regression model and if this requirement is not met as is often the case with less numerous populations, the best option is to use the repeatability model.

Parametri disperzije za veličinu legla svinja

Z. Luković, D. Radojković

Rezime

Veličina legla je jedna od ekonomski najvažnijih osobina, pri čemu je broj živorođene prasadi osobina izbora pri selekciji u cilju povećanja veličine legla u većini odgajivačkih programa u svinjarstvu. Broj živorođene prasadi je pod uticajem brojnih spoljašnjih i genetskih faktora kao i interakcija između njih. Primena metoda mešovitih modela je postala uobičajena za ocenu parametara disperzije metodom maksimalne verovatnoće za ostatak (REML) i za procenu priplodne vrednosti životinja metodom najbolje linearne nepristrasne procene (BLUP). Postoji nekoliko pristupa u odnosu na genetsku ocenu veličine legla. Oni

uglavnom zavise od genetske povezanosti između uzastopnih legala, obima korišćenih setova podataka, računarskih kapaciteta i pouzdanosti podataka. Zahvaljujući jednostavnosti genetska ocena veličine legla je u mnogim zemljama bazirana na modelu ponovljivosti. Niske genetske korelacije među uzastopnim prašenjima su razlog za primenu analize ne više osobina, iako je ova metoda ređe korišćena. Poslednjih godina za ocenu parametara disperzije veličine legla primenjivan je model slučajne regresije. Iako mnoge studije o disperzionim parametrima za veličinu legla uključuju velike farme i moderne rase svinja, povećanje veličine populacije nekih lokalnih rasa svinja otvaraju mogućnost za obnovu odgajivačkih programa tih rasa i za utvrđivanje parametara disperzije za veličinu legla kao osobine koja se uobičajeno evidentira za potrebe menadžmenta na farmi.

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EFFECTS OF FEEDING ON REPRODUCTION IN PIGS

M. Wähner[1], J. Kauffold[2]

[1]Anhalt University of Applied Sciences Bernburg, Germany
[2]Large Animal Clinic for Theriogenology and Ambulatory Service, Faculty of Veterinary Medicine, University of Leipzig, Germany
Corresponding author: m.waehner@loel.hs-anhalt.de.
Invited paper

Abstract: In comparison to previous generations over a twenty year span, the bodyweight of the current animals is much higher and there is increase in the amount of side fat thickness. In all countries with high performance pig production the use of ultrasonic measurement of sidefat thickness in sows and boars is the standard unit of measurement for reproductive capabilities. Essential factors of nutrition for reproduction are vitamins (A, D, E) and minerals (Se, Na, Zn, Cu, Mg, Cr, Fe), but the fat tissue is especially important in its function for reproduction. Generally, feed energy has a key function in the reproductive metabolism. It is the main influence on pancreatic metabolism, similar to the Insulin concentration in blood plasma and the Leptin secretion in fat cells. In sows, four periods during the reproduction process are highly influenced by nutrition level: youth until puberty, endocrinology in estrous cycle, gestation, lactation. The relation between energy, protein, reproduction endocrinology, growth and immune system is complex.

Key words: pig, reproduction, nutrition, body-fat, leptin

Introduction

In recent history, reproductive performance in sows has increased significantly. The genetic trend for litter size of German Landrace and Large White's is rising as shown in Figure 1.

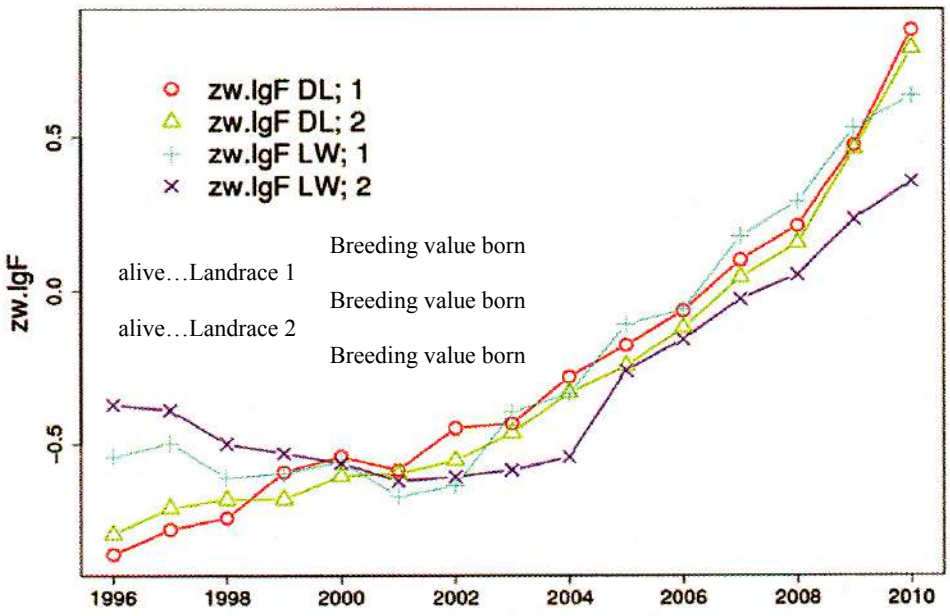


Figure 1. Genetic trend for litter size (born alive per litter) in German Landrace and Large White

The characteristics of the sows have changed from the earlier types. In comparison to previous generations over a twenty year span, the bodyweight of the current animals is much higher and there is increase in the amount of side fat thickness. The body condition is changed as demonstrated in Figure 2.

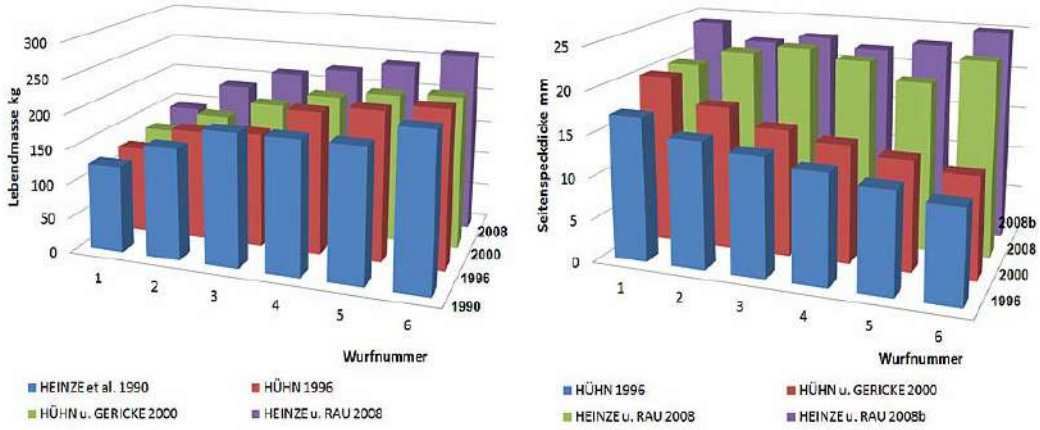


Figure 2. Progress of body condition in sows in the last 20 years

Function of body fat regarding to reproduction

In all countries with high performance pig production the use of ultrasonic measurement of sidefat thickness in sows and boars is the standard unit of measurement for reproductive capabilities. It is international practice to measure only one point 6,5cm of the center back (Figure 3).

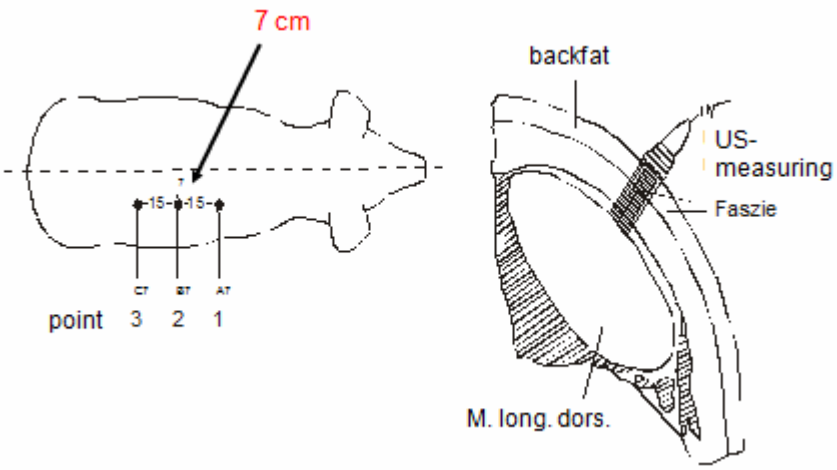


Figure 3. Measuring of backfat thickness with ultrasonic in pigs

It is well-known, that the body fat has an important effect on reproduction physiology. The nutrition level during growth until puberty, during pregnancy and with lactation effects follows general reproductive cycles *(Mullan and Williams, 1989; Weldon et al., 1994)* and depends strongly on feeding levels.
The fat tissue is especially important in its function for reproduction. In the fat, there is a high concentration of 17-ß-estradiol. This is not concentration variant dependent on the cycle. The body-fat has a function as a deposit of estradiol *(Wähner et al., 1993b)*. Additionally there are positive correlations between backfat thickness, concentration of 17-ß-estradiol and the number of ovulated follicles and litter size *(Wähner et al., 1995)*.

Quality of nutrition regarding to reproduction and fertility

Numerous papers confirm the high influence of nutrition on features of reproduction in pigs *(Armstrong et al., 1986; Wähner et al., 1993a; Miller et al., 1997; John and Wähner, 1999; Östein, 2010; Eber et al., 2011)*. *Peltoenimi (2011)*

has demonstrated clearly the effect of feeding intensity on seasonal reproduction performance (Table 1).

Table 1. Effect of feeding intensity on seasonal reproduction performance in sows (*Peltoenimi, 2011)*

	Spring		Autumn	
	ad lib.	restr.	ad lib.	restr.
Litter	2,0	2,78	2,9	2,3
Weaning-estrus interval	5,84	5,06	5,65	6,25
Weaning-pregnancy	5,72	7,71	6,75	13,83
Return%	5,3	11,1	5,0	26,3

The interactions between metabolism and reproduction are varied (Figure 4).

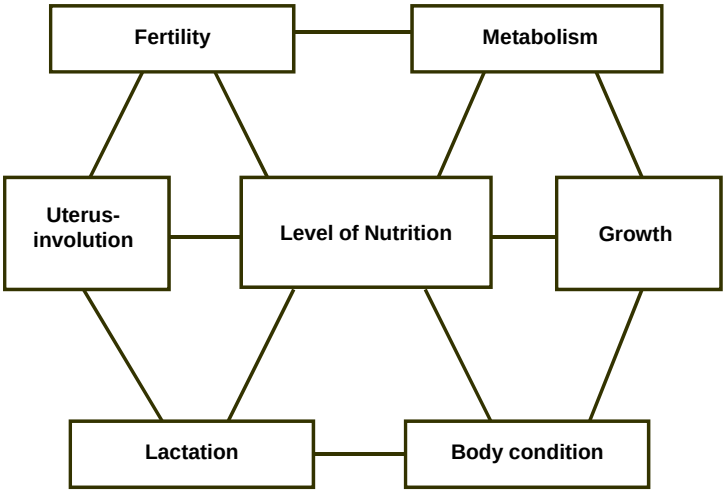


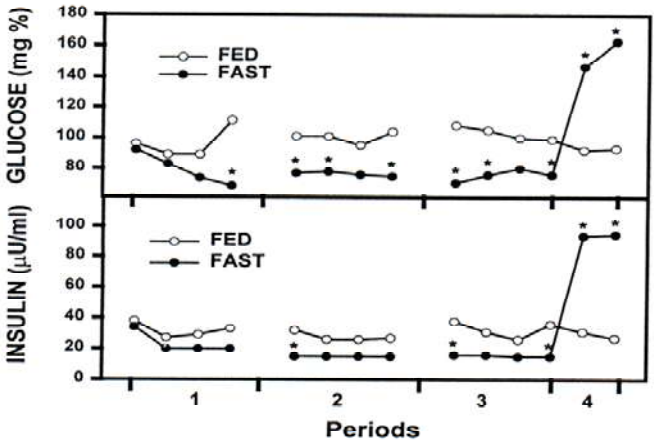
Figure4. Interactions between metabolism and reproduction in pigs

Metabolism has a pervasive and long range effect on all physiological aspects of growth, immunity and most notably reproduction. This is especially critical in high performance sows. An energy shortfall or loss of protein will affect the reproduction processes directly through the inhibition of growth, delayed development of the genital tract, reduced secretion of hormones such as gonadotrophins, gestagens and androgens, delayed puberty, reduced libido and/or reduced estrous behavior.

If there is an absence of energy in blood plasma, the amount of glucose, of insulin and IGF-1(Insulinlike growth-Factor 1) is reduced and the pulsatile

secretion of GnRH and LH are also reduced. In sows, the development of follicles is delayed and the secretion of estrogen in granulosacells is blocked. In boars the secretion of testosterone is reduced and the development of Sertoli-cells and epithels in testes are inhibited.

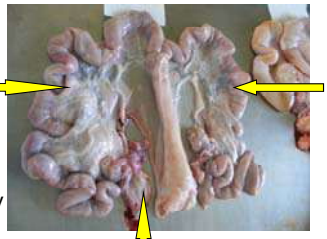
Generally, there is a high sensibility to the level of available energy in pigs. In pre-pubertal gilts, fasting affects the content of Glucose and Insulin directly (Figure 5). When feeding begins again, the content of Glucose and Insulin rise immediately.



Fasting = 28h total no feed
period 1 = 0-4 h, period 2 = 8-12 h, period 3 = 24-28 h after fasting, period 4 = 28-30 h after a new feeding

Figure 5. Influence of fasting on concentration of Glucose and Insuline in prepubertal gilts *(Barb et al., 2001)*

In the peripartal phase of sows, a negative energy balance can affect the physiological processes at the ovaries, in the uterus and the oviduct (Figure 6). This can disturb, interrupt, delay or decelerate special functions. For example the feed intake influences the ovarian follicular development and LH pulsatility (Table 2).



OVARY
- Change in composition of follicle fluid
- Disturbance of oocytes quality & developing potency
- Slow follicle growth
- Delayed ovulation
- Reduced estradiol synthesis
- Function of Corpus luteum (Progesteron)

UTERUS
- delayed involution of uterus
- Prolonged inflammation
- Disturbance of embryonic development (increase of embryonic mortality)

OVIDUCT
- Disturbance of fertilisation and early embryonic development

Figure 6. Possible effects of the peripartal negative energy balance on ovaries and the genital tract in sows

Table 2. Influence of feed intake on ovarian follicular development and LH pulsatility *(Miller et al., 1996)*

	Ad lib.	Restrict.	S.E.M.
Day 13 plasma LH concentration ng/ml	0.44	0.21	0.40**
LH pulses in 12 h on day 13	3.1	0.2	0.53**
Follicles >3mm diameter at weaning	37.2	0.2	4.76***

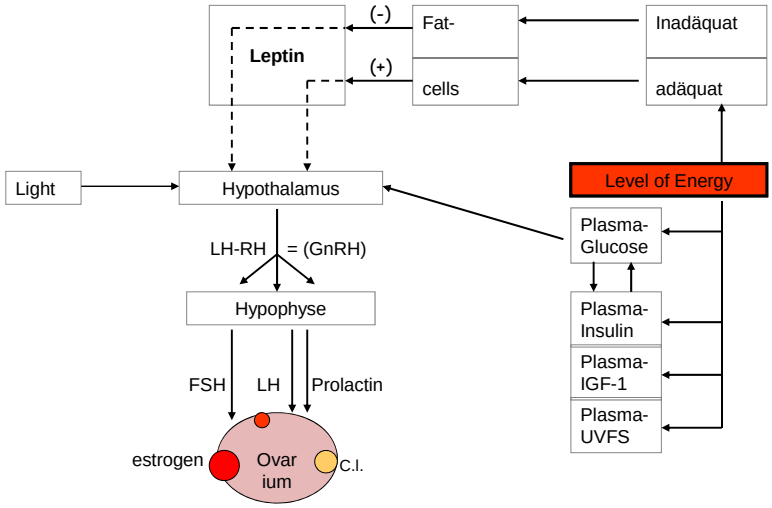
**Treatment difference P<0.01; ***Treatment difference P<0.001

Restricted feeding in early lactation cannot be recommended for early weaned sows and has questionable value for conventionally weaned animals as it is likely to result in reduced subsequent reproductive performance.

Essential factors of nutrition for reproduction are vitamins (A, D, E) and minerals (Se, Na, Zn, Cu, Mg, Cr, Fe). Most of these factors develop special systems for transport in the placenta. If the supply for sows is limited or not sufficient, supply of embryos and fetuses will be preferred. A lack of energy and protein in the last weeks of gestation inhibits the development of muscle and lymphatic tissue in sows. As a result, the abilities of the immune system and extremities are reduced. For reproduction the enervation of immune systems is critically important.

Leptin

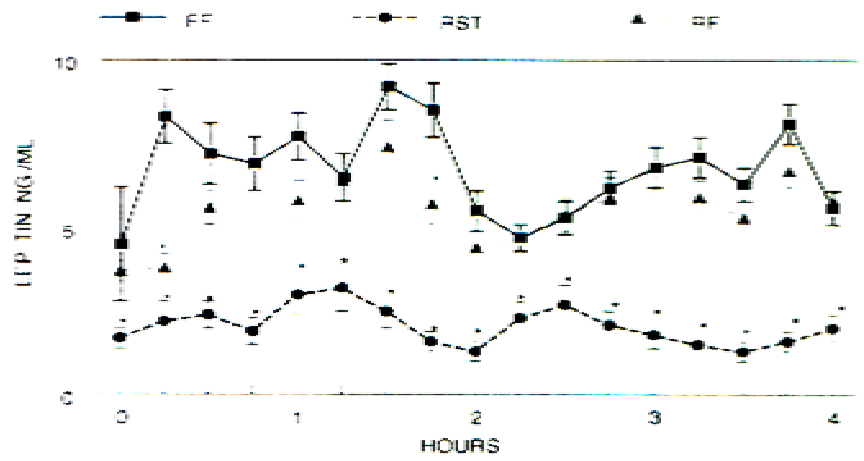
Generally, feed energy has a key function in the reproductive metabolism. It is the main influence on pancreatic metabolism, similar to the Insulin concentration in blood plasma and the Leptin secretion in fat cells (Figure 7). Leptin receptors are found in a lot of tissues, like the brain, heart, bowesl, hypothalamus, pituitary, genitals, kidney, liver, backfat, muscle, bone marrow and the pancreas *(Lin et al., 2000)*.



IGF-I…Insulin-like-growth-factor I, UFS…unsuturated Fatty acids

Figure 7. Regulating influences on cycle on ovaries in female animals *(Ulbricht et al., 2004)*

Of interest is the response time of the Leptin dependent on feed energy level. A feed restriction for 7 days reduced the Leptin concentration about 50% (Figure 8). A conclusion follows that the feed intensity can effect Leptin levels and the resulting reproduction functions immediately.



Restriction 33% of normal ration for 7 days
Investigation at day 7 (time 4 hours)
FF = full fed, RST = restricted fed, RF = refed

Figure 8. Influence of short feed restriction on concentration of Leptin in gilts *(Whisnant and Harell, 2002)*

Leptin has several functions. It is important for the regulation of body weight, influences metabolic energy, and in the Pancreas secretes. Insulin in relation to Glucose metabolism. It maintains normal reproductive functions in sows such as puberty and menstrual cycles. It also influences growth and effects immune reactions *(Schnurrbusch and Kolb, 2004)*.

Leptin influences the onset of puberty. The existence of Leptin is necessary for a critical body weight and to maintain enough body fat. These are presuppositions for puberty. In case of lack of fat, puberty will be delayed. Leptin is the signal for onset of puberty. It is important for maturation of certain regions of brain which are superior of Hypothalamus (Amygdala). Estradiol stimulates synthesis of Leptin. This process depends on age and is most efficient directly before puberty. In front of the background that Leptin stimulates secretion of gonadotrophins, Leptint is responsible for weaning-estrus-intervall. In male pigs testosterone inhibits synthesis of Leptin as a protection for effective and strong fat reducing.

Influence of nutrition on postpartal reproductive physiology

Lactation is a very sensitive time in the reproductive cycle. During this period the uterus and ovaries have to regenerate. This process requires about 3 weeks. A few exogenous influences stimulate regeneration of the reproductive organs, others delay the process. A short regeneration period without disturbances promotes shorter weaning to oestrus intervals. Follicular development is the main prerequisite for ensuring the onset of oestrus in weaned sows.

An experiment has demonstrated the effect of nutrition levels during lactation on the development of genitals of suckling sows and on FSH and LH secretion patterns present until weaning. The objective of the experiment was to investigate the natural response at various times after weaning resulting from different nutrition involving various energy intake levels over a 3 week lactation period. The study was conducted to define the hormonal patterns of both FSH and LH gonadotrophins in primiparous sows fed restricted or ad libitum.

14 primiparous sows were included in two experiments and divided into two groups. The duration of lactation was 21 days generally. The difference between the two groups was the feed ration:

- Group 1:8 sows were given an ad libitum ration with an energy concentration of 14.6 MJ/kg dry matter
- Group 2:6 sows were fed a ration set at 70 % of the feed level of the AL-sows
- Sampling of blood on days 12, 15 and 18 p.p. every 15 minutes starting at 7.00 am for 11 hours with inserted intravenous catheter.
- Analyses for FSH and LH by RIA
- The follicular growth was monitored by daily ultrasonography of the ovaries between day 8 and 20 or 21 p.p.
- Uterine and follicular development was registered in sows after slaughtering at day 1 or 7 after weaning.

The rearing performance of ad libitum fed sows was significantly higher than that of restricted fed sows.

After weaning in ad libitum fed sows the development of the uterus and ovaries was more advanced generally then in restricted fed sows (Table 3).

Table 3. Influence of energy intake during lactation on development of the genitals in weaned sows

Timing of the examination	n	Weight of uterus (g) X — s	Length of uterus (cm) X — s	Ovaries/follicles
sows fed ad libitum (AL)				
24 hours after weaning	3	399 90	98 10,4	Tertiary follicles with diameter: 3-6mm
7 days after weaning	5	478 154		15,4 ± 2,3 Graafian follicles & Corp. haem.
sows fed restricted (RE)				
24 hours after weaning	2	335 175	109 15,0	Tertiary follicles with diameter 3-4 mm
7 days after weaning	4	392 69		13,8 ± 3,3 Corp. haem.

The weight of average uterus was higher. Additionally the development of ovaries and follicular growth at the ovaries was more advanced than in sows after the restricted feeding. The diameter of the follicles in lactating AL-sows had increased (Figure 9). The correlation was r = 0.83. The diameter of the follicles in lactating RE-sows had decreased. The correlation was r = - 0.42.The results show that in sows with a short lactation period energy intake is very important for the development of uterine and follicular growth.

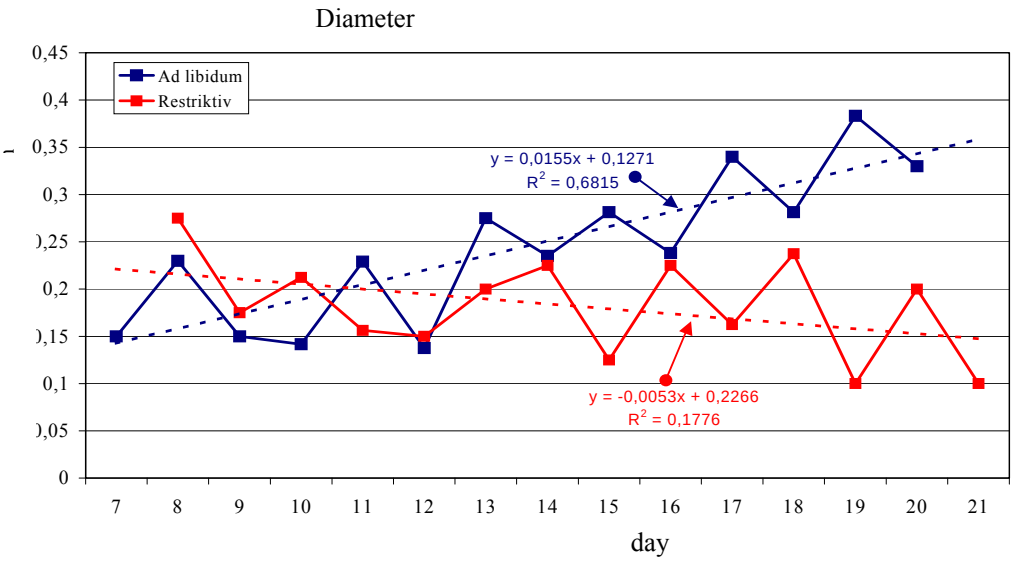


Figure 9. Development of follicles in lactating primiparous sows fed restricted or adlibitum

Following the influence of energy intake in lactating sows on gonadotrophins, secretion patterns were analyzed. Figure 10 demonstrates the concentration of FSH in sows at days 12, 15 and 18 p.p..

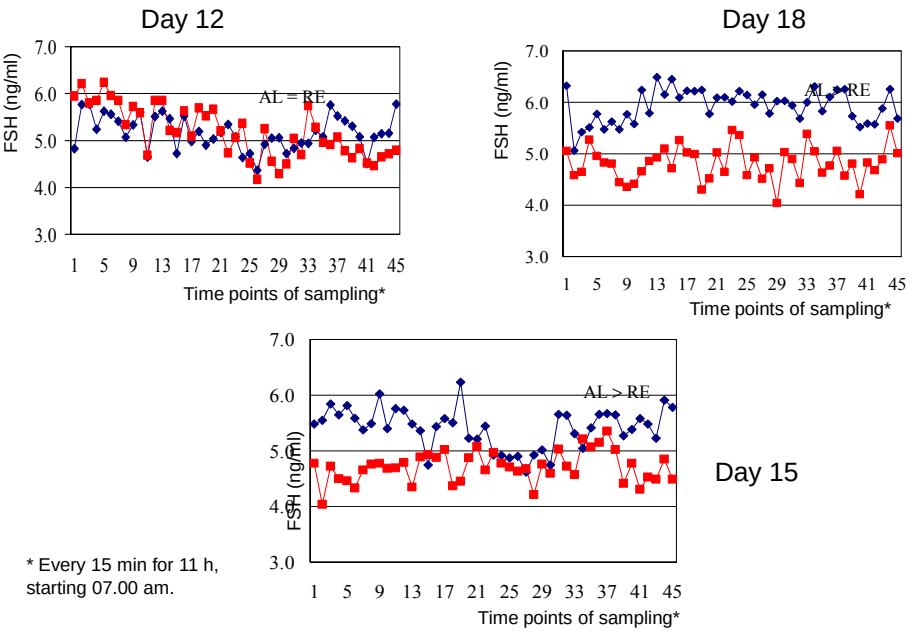


Figure10. Concentration of FSH in frequently taken blood samples of sows fed restricted (n=5) or ad libitum (n=5) diets at different days p.p.

A clear effect of the nutrition level during lactation on FSH-secretion patterns can be seen here. On day 15 pp, the FSH secretion patterns were almost concurring in AL- and RE-sows. A differentiation began on day 15 pp. On day 18 there was a significant difference between the animals of both groups. With the progression of lactation an increased activity of the pituitary and a subsequent increase in the FSH concentration can be observed in the blood.

LH concentrations were significantly higher in AL- than RE-sows at all three bleeding intervalls. This applies to mean LH, Basal-LH number of pulses and LH pulse magnitude (Table 4).

Table 4. LH secretion patterns of sows fed ad libitum (n=5) and restricted (n=5) on different days of lactation

Parameter	Group	Day post partum (±1 day)		
		12	15	18
Mean LH (ng/ml)	ad libitum	1.86 ± 0.02^{aAB}	1.94 ± 0.03^{aB}	1.83 ± 0.03^{B}
	restricted	1.65 ± 0.22^{bB}	1.68 ± 0.02^{bB}	1.79 ± 0.02^{A}
Basal LH (ng/ml)	ad libitum	0.78 ± 0.33^{aB}	1.09 ± 0.31^{aA}	0.67 ± 0.19^{AB}
	restricted	0.11 ± 0.90^{bB}	0.28 ± 0.18^{bAB}	0.62 ± 0.22^{A}
Number of LH pulses (n)	ad libitum	1.00 ± 0.30^{AB}	1.80 ± 0.40^{aA}	1.00 ± 0.50^{B}
	restricted	0.20 ± 0.20	0.20 ± 0.20^{b}	1.00 ± 0.50
LH pulse magnitude (ng/ml)	ad libitum	1.13 ± 0.33^{A}	1.41 ± 0.20^{aA}	0.69 ± 0.32^{B}
	restricted	0.25 ± 0.25	0.26 ± 0.26^{b}	0.78 ± 0.35

Values are given as mean ± SE

Blood samples weren taken every 15 min for 11 h on days 12, 15 and 18(±1day). Values with different superscripts differ significantlywoth a column (a,b) and row (A,B) for the respectivce parameters

In Figure 11 the secretion patterns of LH of lactating sows in three times are shown.

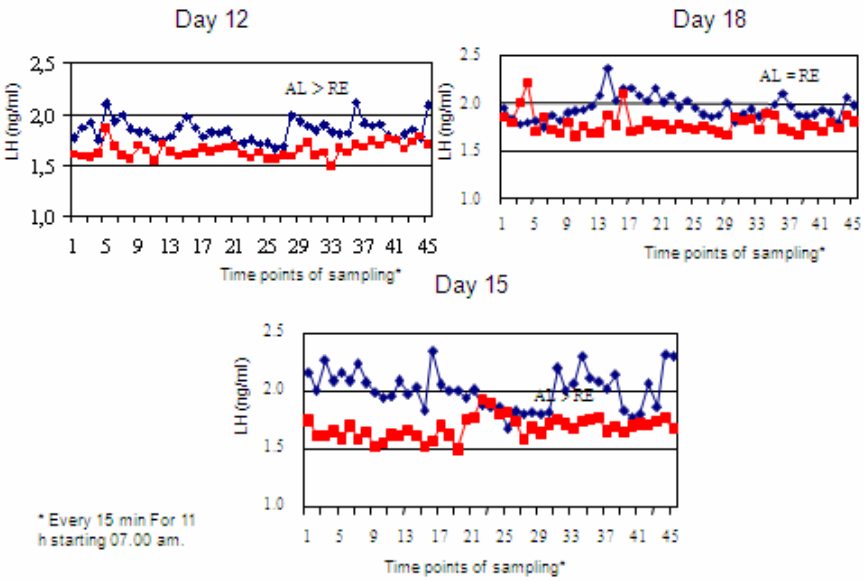


Figure 11. Concentration of LH in frequently taken blood samples of sows fed restricted (n=5) or ad libitum (n=5) diets at different days p.p.

In comparison to FSH the effect of nutrition level during lactation on LH was not so high. The mean concentration of LH is between 1.5 to 2.0 ng/ml. The difference is not significant.

Conclusion

In sows, four periods during the reproduction process are highly influenced by nutrition level:

- Youth until puberty
- Endocrinology in estrous cycle,
- Gestation
- Lactation

In youth, the energy intake influences the growth of uterus and ovaries until puberty. In this time the positive feedback mechanism is to develop for onset of puberty and first ovulation.

The energy level is important for the regulation of hypothalamo-pituitary-adrenal axis (HPA). In male and female pigs, energy deficiency affects growth, puberty, libido, estrus and reduces IGF1. This situation affects pulsate secretion of GnRH and LH with negative consequences for activities on ovaries. Secretion of glucocorticosteroids is increased. Consequently, the concentration of leptin in the fat tissue will be reduced. With the resulting decrease in fat, the amount of 17-ß-estradiol stored is limited with affects to follicle growth and ovulation.

In lactation, restricted feed suppresses follicular development and pulsatile LH secretion at day 14 p.p. in sows. The development of the uterus and ovaries after weaning was delayed by restricted nutrition in lactation. An association to simultaneously observed differences in follicular growth is assumed. FSH concentration during lactation in ad libitum fed sows is higher as in sows fed a restricted diet. Ad libitum fed sows achieved a significantly higher rearing performance then restrictive fed sows. Additionally the amount and kind of crude fiber in feed during gestation influenced the reproduction performance in sows.

This was especially evident in the peripartal phase where a negative energy balance affected processes in the ovaries, oviduct and uterus.

The relation between energy, protein, reproduction endocrinology, growth and immune system is complex.

Uticaj ishrane na reprodukciju svinja

M. Wähner, J. Kauffold

Rezime

U odnosu na prethodne generacije više od dvadeset godina, telesna masa ispod sadašnjih životinja je mnogo veća, a povećana je debljina bočne masti. U svim zemljama sa visokim performansama u proizvodnji svinja, upotreba ultrazvučnog merenja debljine bočne slanine krmača i svinja je standardna jedinica za merenje reproduktivnih sposobnosti. Osnovni faktori ishrane za reprodukciju su vitamini (A, D, E) i minerali (Se, Na, Zn, Cu, Mg, Cr, Fe), ali masno tkivo je posebno važno u funkciji za reprodukciju.

Generalno, energija iz hrane ima ključnu funkciju u reproduktivnom metabolizmu. To je glavni uticaj na metabolizam pankreasa, sličan koncentraciji insulina u krvnoj plazmi i sekrecija leptina u masnim ćelijama. U krmača, četiri perioda tokom procesa reprodukcije su pod velikim uticajem nivoa ishrane: mladost do puberteta, endokrinologije u estrus ciklusa, graviditet, laktacija. Odnos energije, proteina, endokrinologije reprodukcije, rasta i imunog sistema je složen.

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CHARACTERISTICS, STUDIES AND UTILIZATIONS OF CHINESE LOCAL SWINES

Liu Di

Heilongjiang Academy of Agricultural Sciences (HAAS), 368 Xuefu Road, 150086 Harbin, P.R.China.
Corresponding author: liudi1963@sohu.com
Invited paper

Abstract: At present there are 76 local swine breeds in China. According to their geographic distribution and species characteristics, these breeds are divided into 6 types, namely North-China type, Janghai type, Central-China type, South-China type, Plateau type and Southwest-China type. Though there is great characteristic diversity across breeds, all these breeds share remarkable advantages, such as strong fecundity, high stress-resistance and good meat-quality, as well as disadvantages, like poor growth and delayed slaughtering date. By far a lot of studies have been performed on the basic genetic theory of these swine resources. At the same time, by hybridization of different breeds, a variety of new swine strains with new characteristics have been successfully developed. This review will summarize the characteristics of Chinese local swine breeds and the proceedings of study and utilization of these swine breeds in China.

Key word: Chinese local swine, species characteristics, utilization

Introduction

Types of Chinese local swine breeds and their geographic distributions

According to *Chinese Animal Genetic Resource Record • Swine (2011)*, there are 76 local swine breeds, 6 national local-swine-genetic-resource-protection zones, and 40 genetic-resource-protection farms in China. These 76 local swine breeds are divided into 6 types, namely North-China type, Janghai type, Central-China type, South-China type, Plateau type and Southwest-China type, based on their geographic distributions and species characteristics.

North-China type of swine mainly distributes at north of Huaihe River and Qinling Mountain, with representative breeds of Min swine, Eight-eyebrow swine, Hetao big-ear swine, and Hanjiang black swine. This type of swine has tall trunk, narrow back, short and strong limbs, long and straight mouth, large and sagging

ears, longitudinal frontal wrinkles, thick skin, and black hair. They could tolerate cold and roughage. Their meat has more suet, thinner fat, more lean, and thus is tasty. The litter size this swine type is around 12.

South-China type of swine mainly distributes at south of Nanling Mountain and Zhujiang River, with representative breeds of Guangdong swine, Guangxi spotted swine, Guangxi Luchuan swine, and Taiwan Taoyuan swine. This type of swine has small body, sagging back, large drooping belly, thick butt and leg, black or black and white hair, thin skin, thin hair, small head, more frontal wrinkles, and small ears either erected or horizontally stretched. This type of swine was characterized by thin bone, tender meat, thick fat, easy fattening, and high slaughtering rate. The average litter size of South-China type of swine is 8 to 9. This type of swine has 5 to 6 pairs of nipples. Their sexual maturity is relatively early. The sow begins to estrus around 3- to 4-month old.

Central-China type of swine mainly distributes between Changjiang River and Zhujiang River, with representative breeds of Zhejiang Jinhua swine, Huazhong Liangtouwu swine, Hunan Ningxiang swine, Daweizi swine, and Wanzhe spotted swine. Compare to the South-China type of swine, this type of swine has bigger body, wider and more sagging back waist, bigger and more drooping belly. They usually have many frontal horizontal wrinkles, medium large ears which are sagging, black and white thin hair. This type of swine grows fast and matures early. Their meat is tender. The average litter size of Central-China type of swine is 10 to 12. They have 6 to 7 pairs of nipples.

Janghai type of swine mainly distributes between Huaihe River and Changjiang River, with representative breeds of Taihu swine, Jiangsu Jiangquhai swine, Hongqiao swine, and Yang xin swine. There is high diversity of appearances and production performances among different breeds of this swine type. Their hairs are black or black with white spot, and heads are medium large. There are deep wrinkles on forehead showing in diamond shape. This type of swine has big and drooping ears, wide and flat or slightly concave back. This swine type has strong fecundity. Parous sows of this type have average litter size of more than 13 and nipples of more than 8 pairs. This type of swine also sexually matures early, accumulates fat and gains weight faster.

Southwest-China type of swine mainly distributes at Yunnan-Guizhou plateau and Sichuan basin, with representative breeds of Wujin swine, Chenhua swine, Neijiang swine, and Rongchang swine. The features of this type of swine are large head, stubby legs, spin frontal hair and longitudinal frontal wrinkles. Their hair color normally is all black or black with “six white” (nose, tail and limb ends are white), in rare cases is black and white or red. The litter sizes of this swine type are 8 to 10.

Plateau type of swine mainly distributes at Tibetan plateau, with representative breeds of Tibetan swine and Hezhuo swine. This type of swine is small. They have compact body, brawn limbs, long and straight mouth, small and erected ears, narrow and slightly bow back, tight belly, thick skin, and long hair. They sexually mature late. The litter size of this swine type is 5 to 6. They generally have 5 pairs of nipples.

The above descriptions of Chinese local swine can be summarized as "north big south small" in body size, "north black south versicolor" in hair color. As far as litter size is concerned, it is the biggest in Taihu swine which distributes along Changjiang River, and is gradually smaller to north, south, or west geographically *(Hongquan and Moyou, 1999)*.

Genetic Characteristics of Chinese local swine

Strong Fecundity

Chinese local swine generally has strong fecundity, which is shown as early maturation of sow, high ovulation rate and big litter size. Researches on Erhua-face swine, Jiaxing black swine, Jiangquhai swine, Neijiang swine, Dahuabai swine, Min swine, Jinhua swine, Daweizi swine, Hetao big-ear swine showed that the average puberty of these swine breeds is 98.08 ± 9.7-d old, ranges from 64-d (Erhua-face swine) to 142-d (Min swine). The average body weight is 24.30 ± 3.5 kg, ranges from 12.22 kg (Jinhua swine) to 40.5 kg (Neijiang swine).
Chinese local boar also shows high fertility, which is displayed as quick weight-gain of their testis. Their testes start to grow rapidly around 60-d old. At 90-d old, the average weight of their testes reaches 28.96 ± 3.57 g, and at 120-d old, the average weight increased to 48.08 ± 5.23 g. The testes weight of Erhua-face boar reaches 159.7 g when it is 180-d old. Even when it is 90-d old, the weight reaches 40.4 g, which is similar as that of Landrace swine when it is 130-d old.

The average first-mating age of Chinese local swine is 128.57 ± 4.46-d old, which is much earlier than 210-d of large Yorkshire swine. At the first-mating age, the average level of testosterone in Chinese local swine is 372.3 ± 69.1 ng/mL. It is as high as 466.88 ng/mL in Jiaxing black swine and 488.15 ng/mL in Dahuabai swine, which are 5 times of the 95 ng/mL in large Yorkshire swine. The above data show that Chinese local boar develops testicular function very fast, which directly leading to their early sexual maturity *(Yong and Qing, 1999)*.

Strong resistances

Resistance to cold or hot

North-China local swine shows strong resistance to cold, with Min swine as an outstanding representative. When Min swine was cultured over a year under atmospheric temperatures as low as -20°C and as high as 32°C, the change of its body temperature (anal temperature) is only as small as 0.88°C. The change of its heartbeat during this period is only 0.41 beats per minute, which is 50 times smaller than the 21.8 beats per minute of the Harbin white pig.

At high temperature, increasing breath is the main way for swine to regulate their body temperature. Thus the increase of breath indicates their adaptability to high temperature, the less, the better. When temperature increased from 22.5°C to 32°C, the breath of Harbin white swine increased 47.94 times per minute, and that of Min swine increased 31.87 times per minute. When temperature was artificially changed from 27°C to 38°C, the breath of Dahuabai swine and Landrace swine increased 52.6 and 60.8 times per minute, respectively.

Resistance to starvation

Under the artificially-controlled low-level breeding condition (30 days fully meet needs, and then 30 days meet two-thirds needs), Min swine tolerated longer than Harbin white swine. Erhua-face swine kept gaining weight under low-level breeding. Its weight-gaining is more than 2 times of that of Landrace swine under the same condition.

Adaptability to high altitude

Studies indicated that the blood physiological compensatory of Neijiang swine showed distinct adaptability to high altitude from general pig breeds. When moved from an altitude of 500 m of Chengdu region to an altitude of 3,400 m of Ganzi Tibetan autonomous prefecture, the main blood physiological and biochemical indicators of Neijiang swine, such as red blood cell number, hemoglobin, and serum gamma globulin content were significantly increased. This has great benefits to adapt the thin air high altitude environment. On contrary, Landrace swine did not show good adaptability under the same conditions and kept getting diseases and dying.

Good Meat Quality

The meat of Chinese local swine breeds generally has the following features:

1. Normal red color, no PSE meat. Score 3 or 4 points for most local swine breeds. pH of back longest muscle is above 6.0 45 minutes after slaughter.
2. Good water retention. Average water loss rate for Min swine is 20.42% ± 1.61%, for Jiangquhai swine is 12.77% ± 0.05%, for Erhua-face swine is 10.80% ± 1.85%, and for Jiaxing black swine is 11.26% ± 0.05%, averagely 3.8% lower than foreign swine.
3. Muscle marbling is moderate. On a 5-point scale, mostly scored 3 (moderate) or 4 (much), never scored 1 (very low) or 2 (very few).
4. Smaller muscle fiber diameter. According to determination on 9 swine including Min swine, the average muscle fiber diameter is 42.28 μm, 16.71% smaller than that of introduced swine. Muscle fiber number per area unit is averagely 26% more than that of the control group.
5. High intramuscular fat content. Chinese local swine intramuscular fat content was 4.97% on average, 2.23% higher than introduced pig. More meaningfully, the unsaturated fatty acid content in back longest muscle of some swine is higher than general pig. For example, it is 67.47% in Gaoligong mountains swine and 59.31% in Bao shan swine, compare to 55.83% in general pig.

Slow growth, special development regularities

Slow growth is another feature of the Chinese local swine. For example, from 60-d to 300-d, the daily weight-gain of Erhua-face swine and Dahuabai is 385 g and 343 g, respectively. From 75-d to 250-d, the daily weight-gain of Min swine is 418 g. These are much lower than foreign swine breed. Low birth weight and slow postnatal development are all important factors leading to slow growth of Chinese local swine.

Chinese local swine has the characteristics of early maturity, high fat meat rate, thick skin, and small slaughtering weight. The fat meat rate reaches 1:1.5 in Jinhua swine at 55 kg stage, Dahuabai swine at 65 kg stage, Neijiang swine at 70 kg stage in carcass meat, while that of Landrace swine at 90 kg stage only reaches 1:2.4. When Chinese local swine grows to 90 kg, their lean rate normally reaches about 40%, and fat rate is lower than 40%. The strong intra-abdominal fat storage ability is one of the reasons that lead to more feed consumption.

The most worth mentioning resource of Chinese local swine is miniature (or micro) swine. China has rich, good quality miniature swine resources, like Xiang swine, Tibet swine, Wuzhishan swine and Taiwan Lanyu small-ear swine.

The weights of adults of these swine breeds are mostly in 40 to 45 kg, smaller than foreign miniature swine, such as Minnesota-Hormel miniature swine (adults weigh from 70 to 90 kg weight).

Chinese local swine use & research

Genetic Basis

Jinyong Wang's team from Chongqing Academy of Animal Husbandry and Xuewei Li's team from Sichuan Agricultural University worked together on Rongchang swine and obtained remarkable achievements, mainly including: Constructed the first BAC library of Rongchang swine; Found the early growth regularity of muscle and adipose tissue; Obtained the gene expression profile in different parts of the adipose tissue; Explored many new microRNA; Studied the genetic mechanisms of deaf; Built the Rongchang swine digital model; Developed two specialized strains; Eventually established the level 3 protection system for Rongchang swine genetic resource.

Di Liu's group at Heilongjiang Academy of Agricultural Sciences has done systematically study on Min swine breed, mainly including: Genetic mechanisms of high fertility; Cold resistance mechanism; Meat quality traits, production performance, physiological and biochemical index determination; Whole-genome sequencing and hybridization of different combinations.

Bang Liu's team at Central China Agriculture University focuses on Tongcheng swine, mainly including: Meat characteristics and corresponding genetic basis; Genetic basis of disease resistance; Genetic basis of the color; New breed development (with large Yorkshire swine hybridization).

Utilization by Hybridization

Develop new strain

1. Use local swine breeds to culture new strain, like the long-body strain and fast-grow strain developed from Dahuabai swine; Strain I (fast-grow), strain II (long-body), and strain III (plump) developed from Jinhua swine; Fast-grow strain developed from Ningxiang swine.
2. Hybrid local swine breeds with exotic swine to get new strain. For example, cross between Qingping swine and Duroc swine to get D-Qingping strain; Cross between Guangxi-Guangdong Xiaohua swine and Landrace swine to get Guangxi-Guangdong Hua strain.

3. Develop newer strain from identified new breed, such as Xinhuai swine new strain, Harbin white swine new strain, Gansu black swine lean I strain.
4. Hybrid identified new breed with exotic swine, such as Hubei white swine strain VI, Sichuan white swine strain II, Xinwenzhou white swine strain, Gansu black swine lean strain II, Shanghai white swine (Bao strain) lean strain, Xinjiang white swine lean swine new strain type 21.
5. Inbred strain or closed group of Chinese miniature swine to make experimental animal, such as Wuzhishan swine inbred strain, Guizhoujianhe swine II strain, Banna miniature swine inbred strain and Hainan Wuzhishan swine inbred strain *(Jing and Song, 2000)*.

Breed new breed

Since 1997, National Animal Breed Authorized Committee authorized 9 new swine breed, namely Nanchang white swine, Army 1 swine, Sutai swine, Yunan swine, Dianlu swine, Dahewu swine, Wulaihei swine, Luyanbai swine, Songliao swine *(Zhilian, 2002)*.

Breed specialized strain

Nation authorized 3-line-hybrid specialized strains using local swine are: Shennong swine specialized strain, Tianfurou swine specialized strain, Yihebai swine specialized strain, Diansa swine specialized strain, Lu farm 1 swine specialized strain, Yurong 1 swine specialized strain, Guangming swine specialized strain (2 line hybrid), Huannong wenshi swine specialized strain (4 line hybrid), and Yu swine specialized strain 1 (4 line hybrid).

Conclusion

China is not only a swine meat producer, but also a swine meat consumer, at the same time it is a diversified market. People in China have different requirements for swine meat quality. They want not only the meat with high lean rate, like the meat of imported three-line-hybrid swine, but also the high quality meat with high intramuscular fat content. As a consequence, to produce high quality meat by breeding local swine drew more and more concern of national and local governments. At the same time, the excellent characteristics of Chinese local swine, like high resistance and miniature, also attracted more and more attentions from domestic and foreign researchers, we believe that these traits will be utilized well in future.

Karakteristike, ispitivanje i korišćenje kineskih lokalnih rasa svinja

Liu Di

Rezime

Trenutno postoji 76 lokalnih rasa svinja u Kini. Prema njihovoj geografskoj distribuciji i karakteristikama vrste, ove rase su podeljene u 6 tipova, odnosno severno-kineski tip, Janghai tip, centralno-kineski tip, južno-kineski tip, nizijski tip i jugozapadni kineski tip svinja. Iako postoji velika raznolikost karakteristika rasa, sve ove rase dele značajne prednosti, kao što su jaka plodnosti, visoka otpornost na stres i dobar kvalitet mesa, kao i nedostatke, kao što su spor rast i kasniji termin klanja. Do sada su sprovedena mnoga istraživanja sa stanovišta osnovne genetske teorije ovih resursa svinja. U isto vreme, hibridizacijom različitih rasa, uspešno je razvijen niz novih sojeva sa novim karakteristikama. Ovaj pregled će sumirati karakteristike kineskih lokalnih rasa svinja i postupak istraživanja i korišćenja tih rasa svinja u Kini.

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PORK PRODUCTION AND EVALUATION OF MEAT YILED IN SERBIA AND THE WORLD

Č. Radović[1], M. Petrović[2], B. Živković[1], D. Radojković[2], N. Parunović[3], R. Savić[2], M. Gogić[1]

[1]Institute for Animal Husbandry, 11080, Belgrade-Zemun, Republic of Serbia
[2]Faculty of Agriculture, University of Belgrade, 11080, Belgrade - Zemun, Republic of Serbia
[3]Institute of Meat Hygiene and Technology, 11000, Belgrade, Republic of Serbia
Corresponding author: cedomirradovic.izs@gmail.com
Invited paper

Abstract: In this paper, the importance of pig production in livestock production in R. Serbia and globally are presented considering the high share of pork in total meat production. The biggest producer of pigs and pork is China. In year 2011, 48.9% of total pig population in the world was produced in China. In the same year, in the United States, 6.89% and in Brazil 4.08% of the total number of pigs in the world were produced. The greatest decrease of the pig population from in 1999 until 2011 was recorded in Hungary, the Czech Republic and Bulgaria. The share of Serbia in the total number of pig population in the world in 2011 was 0.39%. The highest pork production in 2011 was in China, and it accounts for 46.84% of total meat production, followed by the United States (9.39%), Germany (5.10%), Spain (15.3%) and Brazil (2.93%). Participation of Serbia in this regard in 2011 amounted to only 0.25% in relation to the total production of pork in the world. The share of pork of 54.4% of the total production of all kinds of meat in our country makes it the most important branch of livestock industry.

Key words: production of pigs, meat yield evaluation, assessment method

Introduction

Pig production in Serbia, since ancient times is important branch of agricultural production. The importance of pig production in livestock production and the overall economy of Serbia comes from its economic and biological importance. The economic importance stems from the fact that production of pigs is the main source of meat and meat products. The share of pork in the total meat production in the Europe is over 50%, and Asia over 58%. In our country, pork production is of great importance. The share of pork from 54.4% of the total production of all kinds of meat in our country makes it the most important

livestock industry branch *(Radović et al., 2011)*. In developed countries, pig farming is an activity that has the characteristics of industries with high specialization in terms of selection and breeding of breeding material on the one hand and the production of fatteners on the other. Selection and breeding of pigs in the world is taken over by multinational breeding companies (breeding company) which use highly sophisticated knowledge of geneticists and laboratory equipment - molecular, population, and quantitative genetics and biometrics, to create nucleus genotype breeds, hybrids and lines, that are subsequently marketed for very high profits and for the multiplication and use in the production (as required by the market) certain schemes for use are given, which is usually a diagram of the hybrid breeding program, but in which the nucleus is under the full authority of a breeding company *(Radović, 2013)*. In regard to the total number of pigs, 62% are found on farms in Central Serbia, and 38% of farms in Vojvodina *(Popović et al., 2010)*. The genetic potential of the majority of individual farms is unsatisfactory. For this reason the total production of fattening pigs realize improved costs of food with lower percentage of lean meat *(Zekić et al., 2008)*. In addition to slaughterhouse products which are used in human consumption and by-products of other industries, a very important and valuable by-products of swine production is manure. All that is not adopted by the body is excreted in feces and urine with or without litter, scattered food, dust, hair, etc., are a by-product or manure. Manure is a complex organic compound that can be used to produce biogas and as a valuable organic fertilizer which, if used according to the principles of good agricultural practices, can contribute to repairing, maintaining and increasing the productive capacity of agricultural land, and if it is not managed according to the principles of good agricultural practice is very serious and dangerous environmental pollutant.

Production of pigs and pig meat

Table 1 shows the pig population in the world and some countries (countries that are the largest growers and the state with the largest fluctuations in the production of pigs) for the period 1999 to 2001 and from 2003 to 2005 and since 2007 until the year 2011. The data presented shows that China is the largest grower of pigs in the world. Of the total number of pigs in the world, in year 2011, China produced 48.9%. In the same year, in the United States, 6.89% and in Brazil 4.08% of the total number of pigs in the world were produced. The share of Serbia in the total number of pig population in the world in 2011 was 0.39%. For these countries (Table 1) 2007 through 2009, the total number of pigs has increased in China, the U.S., Brazil and Vietnam. The upward trend in the 2011 was retained only in Brazil, where as in the United States and Germany, there was a reduction in the number of pigs in 2010, and in China in 2011. The greatest decline of the pig

population from in 1999 until 2011 was recorded in Hungary, the Czech Republic and Bulgaria.

Table 1. Pig population in the World (in 000)

Country	1999-2001	2003-2005	2007	2008	2009	2010	2011
China	430.971	424.718	425.265	446.463	451.178	476.267	470.961
USA	60.229	60.324	62.516	65.909	67.148	64.925	66.361
Brazil	31.669	33.151	35.945	36.819	37.000	38.957	39.307
Germany	25.904	26.284	27.125	26.687	26.887	26.509	26.758
Spain	22.079	24.612	26.061	26.026	26.290	25.343	25.635
Viet Nam	20.293	26.154	26.561	26.702	27.628	27.373	27.056
Mexico	16.473	15.048	15.500	16.100	16.100	15.435	15.547
Hungary	5.216	4.685	3.987	3.871	3.383	3.247	3.169
Czech R.	3.761	3.122	2.433	1.917	1.909	1.908	1.749
Bulgaria	1.459	987	1.013	889	784	730	664
Belarus	3.565	3.341	3.642	3.598	3.704	3.781	3.887
Serbia	4.031*	3.432*	3.832	3.594	3.631	3.489	3.287
World total	**892.592**	**900.246**	**921.935**	**937.954**	**941.213**	**967.616**	**963.044**

* Serbia and Montenegro; Source: http:// faostat.fao.org

Table 2 shows the production of pork in the world and some countries for different periods (1999-2001 and 2003-2005) and years (2007 to 2011). The greatest pork production in year 2011 was recorded for China, and it accounts for 46.84% of total meat production, followed by the United States (9.39%), Germany (5.10%), Spain (15.3%) and Brazil (2.93 Participation of Serbia in this regard in 2011 amounted to only 0.25% in relation to the total production of pork in the world.

Table 2. Production of pork (in 000 tons) - Source: http:// faostat.fao.org

Country	1999-2001	2003-2005	2007	2008	2009	2010	2011
China	40.769	44.845	43.933	47.190	49.879	51.681	51.535
USA	8.682	9.247	9.951	10.599	10.442	10.186	10.331
Brazil	2.546	2.990	2.990	3.015	2.924	3.195	3.227
Germany	4.053	4.354	4.985	5.111	5.277	5.488	5.616
Spain	2.929	3.145	3.439	3.484	3.291	3.369	3.469
Viet Nam	1.414	2.032	2.553	2.470	2.553	3.036	3.099
Mexico	1.027	1.068	1.152	1.161	1.163	1.175	1.202
Hungary	598	501	499	461	453	452	435
Czech R.	428	406	360	336	300	291	275
Bulgaria	249	74	76	72	74	70	72
Belarus	305	307	372	376	388	398	419
Serbia	617*	558*	289	266	252	269	271
World total	**90.137**	**97.108**	**100.165**	**103.983**	**106.069**	**109.370**	**110.012**

* Serbia and Montenegro; Source: http:// faostat.fao.org

In Serbia, in 2010, the population was 7.291.436. In the same year a total of 472.000 tons of meat was produced (beef, pork, mutton, and poultry). Of the total amount of meat produced in Serbia, share of pork was 57% or 269.000 tonnes *(Statistical Yearbook of the Republic of Serbia, 2011)*, i.e. 36.9 kg of pork per capita.

Assessment of meatiness/meat yield

In the evaluation of leanness *in vivo* or *in vitro*, the total work in the field of genetics, selection, nutrition, reproduction, and health care is estimated. Knowledge of the variability of production traits of breeding animals is a requirement for working on genetic improvement of the quality of pigs. For this purpose, tests are carried out and recorded data for all the properties in respect of which the selection is performed. Efficiency in swine production is estimated based on three indicators: annual productivity of sows, feed conversion and meat yield/lean meat in animals. Quantitative and qualitative carcass traits depend on the selection methods *(Radović et al., 2012a)*. It is known that some quantitative traits of pigs are unequally inherited, which means that the opportunities for improving the selection vary. The coefficients of heritability for growth traits and carcass quality are moderate to high *(Hermesch et al., 2000; Gorjanc et al., 2003; Radović et al., 2003; Petrović et al., 2006)*. Intensified selection for meat yield causes a significant reduction of subcutaneous fat *(Bahelka et al., 2007)*. Breeding value of young animals can be used for assessing the breeding value of the parents, and therefore choose the best combination of parents for breeding, and thus provide a significant increase in the production and quality of pork in the country *(Jukna et al., 2009)*. Assessment of carcass and meat quality is very important for breeders of pigs and pork processors. It is known that the meat industry is supplied with raw material for products of uneven quality. Timely collection of feedback from the slaughter line and/or processing industry allows breeders to assess the effects of breeding selection work and make changes in the future work if there are requirements by the slaughter and meat industry and to achieve greater genetic gain of medium and high heritable properties *(Petrović et al., 2004)*.

Assessment of meatiness/meat yield *in vivo*

Evaluation of leanness *in vivo*, performance or direct test in our country, is the main tool for the study of offspring, candidates for further selection and reproduction. In implementing this method of testing the primary interest is focused on growth, feed efficiency (conversion) and carcass traits (backfat thickness, surface and/or depth of back muscle *m.longissimus* (ML). Genetic

improvement in the selection of the phenotype depends on the heritability of traits and selection intensity. Given that the performance test covers medium to high heritable traits, the improvement of these traits in offspring can be expected.

In our country, boar performance test is carried out on the farm (individually or collectively housed animals) and of gilts (group housing) on the farm. Central test stations provide a number of advantages compared to on farm testing. One of them is the need to provide greater standardization (control) of the test environment and improve the testing procedure by minimizing the potential effects of uncontrolled effects. In Serbia we do not have any test station so this is one of the drawbacks to improve pig production. At the end of the testing, back fat thickness and depth of the ML are measured, depending on the ultrasound apparatuses that are being used for that purpose. In our country currently used devices are Krautkrämer, Piglog 105, Renco, Sonomark SM-100 and Aquila vet (Figures 1, 2, 3, 4 and 5).

Figure 1. Krautkrämer
(Foto: Č. Radović)

Figure 2. Piglog105
(Foto: Č. Radović)

Figure 3. Renco
(Source:www.rencocorp.com)

Figure 4. SonoMark SM 100
(Foto: Č. Radović)

Figure 5. Aquila vet
(Foto: Č. Radović)

In recent years, the possibility of using ultrasound devices for the measurement of carcass quality in live animals and carcasses after slaughter has been examined. It

is considered necessary to carry out inspection and testing of equipment on a representative sample of at least 120 carcasses that describe the population of pigs. Regardless of who will use ultrasound, it is very important that each of them meets a standard that applies to the value of the coefficient of determination (R^2>0.64) and residual standard-deviation (RSD <2.50).

Assessment of meatiness/meat yield *in vitro*

The first Yugoslav standard for meaty pigs for industrial processing JUS E.CL.021 was passed in 1969. year. It began to be applied in the control and estimation of carcass lean meat at the slaughter line in almost all slaughterhosues in 1973. year. Minor changes were made in 1985, and since then, the use of Regulation on the quality of slaughtered pigs and pork categorization *(OG SFRY, 1985)*.

This Regulation stipulates the minimum requirements in terms of quality of meet (pork) in carcasses, carcass sides and carcass parts/cuts, in basic and edible parts of slaughtered animals, as well as housing conditions, storage, packaging and transportation of meat and edible parts. According to the providions of the Regulation, the meatiness of pork carcass or carcass sides means the total mass of muscle tissue without the meat of the abdominal-rib part and head. Meatiness of carcass sides of meaty pigs is determined on the slaughter line, at least 1 hour after slaughter - the mass of warm carcass and thickness of the back fat are measured. Backfat tissue, with the skin, is measured in the middle of the back, where the fat is the thinnest (intercostal space between the 13th and 15th dorsal vertebra) and the lower back at the place where the muscle *m. gluteus medius* grows into the fatty tissue. The sum of these measures is the back fat thickness. To determine the yield and the share of meat in carcass sides of meaty pig on the basis of the measurements, the tables are used that are integral part of the Regulation.

In our literature *(Radović et al. 2009; 2010; 2012b; Radović 2013)*, negative experiences have been presented in use of the above mentioned Regulation, indicating that there is a conflict between the meat yield in the carcass of slaughtered pigs expressed in kilograms and meat yield expressed percentages. It is likely the cause of the termination of determining the share and yield of meat in carcass sides on the slaughter line in Serbia. Given the deficiencies presented of our Regulation, in the countries of the former Yugoslavia, that Regulation is amended (the Republic of Slovenia in 1995, and in the Republic of Croatia 1999.) and harmonized with current EU regulations. All outlined imposes the need for harmonization of our legislation with the standards of developed countries, especially the EU and the introduction of modern methods of evaluation of carcasses quality on the pig slaughter lines, with mandatory application.

There are a number of automatic devices used to determine the leanness on the slaughter line. In Figures 6, 7, 8, 9, 10 and 11 automatic devices for determining the meat content on the slaughter line are presented.

Figure 6. Hennessy Grading Probe (HGP)
(Source: www.hennessy-technology.com)

Figure 7. Auto Fom
(Source: www.carometec.com)

Figure 8. Fat-O-Meat'er (FOM)
(Source:www.carometec.com)

Figure 9. Fat-O-Meat'er II (FOM)
Source:www.carometec.com

Figure 10. UltraFom 200
(Source: http://www.carometec.com)

Figure 11. UltraFom 300
(Source: http://www.carometec.com)

For each of these devices and the method of "two points" in R. Czech Republic there is a formula to estimate lean meat (EC Decision No 1/05, 2005) in pigs with a hot carcass weight of 60-120 kg (Table 3).

Table 3. Description of methods and evaluation of leanness in Czech Republic

Method	Description	Formula
"Two point" (Zwei-Punkte, ZP)	It is determined by measuring fat thickness with skin in millimeters on the lower back, at its thinnest point, and where *m. gluteus medius* grows into fat tissue (ZP) and the depth of *M. longissimus* measured as the shortest connection of the anterior (cranial) end of *m. gluteus medius* to the upper (dorsal) edge of the spinal canal M (ZP).	ý=49,62542–0,63371 S(ZP)+0,23525 M (ZP)
Fat-O-Meater (FOM)	It is determined by measuring the thickness of backfat (FOM) with skin in millimeters, 6.5 cm from the medial line between the 2nd and 3rd last rib and ML muscle depth M (FOM) at the same time in the same place as S (FOM).	ý = 59,86131–0,72930 S (FOM)+0,12853 M (FOM)
Hennessy Grading Probe (HGP 4)	It is determined by measuring the thickness of backfat (HGP) with the skin in millimeters, 7.5 cm from the medial line between the 2nd and 3rd last rib and ML muscle depth M (HGP) at the same time and the same place as S (HGP).	ý = 61,34154 – 0,81609 S (HGP)+0,12901 M (HGP)
Ultra-FOM 300	It is determined by measuring the thickness of backfat (UFOME) with skin in millimeters, 7 cm from the medial line between the 2nd and 3rd last rib and ML muscle depth M (UFOME) at the same time and the same place as S (UFOME).	ý =64,64865–0,76656 S(UFOM)+0,06425 M (UFOM)

Conclusion

Development and improvement of pig production is strongly dependent on the economic situation in the country and therefore the world economic situation of livestock in general, and in any case of certain state/government economic measures that can accelerate the development of this branch of farming.

Also, improved market conditions for pig meat and meat products should be considered. Most of the pig production is focused on providing meat for human consumption. The use of artificial insemination, and thus obtaining a large number of progeny per boar, the need is imposed for selecting boars on the basis of their genetic potential in producing more offspring that will have a greater life gain, better feed conversion and higher proportion of meat in the carcass as and a commercially desirable meat quality. Pig breeder must choose parental pairs (sows and boars) to give offspring with traits which correspond to the wishes of consumers. It is necessary to re-establish central boartest station, given that with the use of artificial insemination not only the more rational use of boars is

achieved, but also greater selection effect. Annually, from one high quality boar, 2.500 to 3.000 doses of semen can be obtained, and a large number of offspring. Based on this data we see that selecting and reproducing quality breeding boars, which in terms of genetic material correspond to selection/breeding objective, increase the frequency of desirable genes, thereby significantly faster changing the genetic basis of herd or population. The central test station would facilitate greater standardization (control) of the test environment and improve the testing procedure by minimizing the potential effects of uncontrolled effects and obtain an impartial assessment of breeding values for each test boars.

Also, it can be said that we are one of the few, if not the only country in Europe where the unclassified carcasses of pigs are marketed, with all the negative consequences for our pig production, but also the meat industry.

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Proizvodnja svinjskog mesa i procena mesnatosti u Srbiji i svetu

Č. Radović, M. Petrović, B. Živković, D. Radojković, N. Parunović, R. Savić, M. Gogić

Rezime

Razvoj i unapređenje svinjarstva dosta zavisi od ekonomske situacije u zemlji i svetu samim tim i ekonomskog položaja stočarstva u celini, a u svakom slučaju i od određenih ekonomskih mera države koje mogu da ubrzaju razvoj ove grane stočarstva.

Takođe treba računati i na poboljšanje tržišnih uslova za svinjsko meso i proizvode od mesa. Najveći deo svinjarske proizvodnje danas je usmeren na obezbeđenje mesa za ljudsku potrošnju. Upotrebom veštačkog osemenjavanja krmača, a samim tim i dobijanjem velikog broja potomaka po jednom nerastu, nameće se potreba za odabirom nerasta na osnovu njihovog genetskog potencijala u proizvodnji većeg broja potomaka koji će imati veći životni prirast, bolju konverziju hrane i veći udeo mesa u polutkama kao i da imaju komercijalno poželjan kvalitet mesa ("poželjan" znači prihvatljiv kvalitet za potrošača).

Naravno, svaki odgajivač svinja, takođe, treba da poznaje šta njihov kupac želi, tako da mora izabrati roditeljske parove (krmače i neraste) koji će dati potomstvo sa osobinama koje odgovaraju željama potrošača. Potrebno je da se ponovo formira Centralna testna stanica za nerastove. Obzirom da se primenom veštačkog osemenjavanja osim racionalnijeg korišćenja nerasta ostvaruje i veći selekcijski efekat. Godišnje se od jednog kvalitetnog nerasta može dobiti 2500 do 3000 doza semena, odnosno veliki broj potomaka. Na osnovu ovog podatka vidimo da odabiranjem i reprodukovanjem kvalitetnih priplodnih nerasta, a koji u pogledu nasledne osnove odgovaraju cilju selekcije, povećavamo frekvenciju poželjnih gena i time znatno brže menjamo naslednu osnovu zapata, odnosno populacije. Centralna testna stanica bi omogućila veću standardizaciju (kontrolu) testnog okruženja i unapređenja procedure testiranja putem minimiziranja dejstva potencijalnih nekontrolisanih efekata i dobijanje nepristrasne ocene priplodne vrednosti za svakog testiranog nerasta.

Takođe, može se reći da smo jedna od retkih, ako ne i jedina zemlja u Evropi u kojoj se u prometu nalaze neklasirane polutke svinja, sa svim negativnim posledicama po naše svinjarstvo, ali i industriju mesa.

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EXAMINING THE EFFICIENCY OF THE PARTIAL SUBSTITUTION OF THE MAIZE WITH A BY-PRODUCTS OBTAINED BY MANUFACTURING VEGETABLES AND FRUITS IN MIXTURES FOR WEANED PIGLETS

G. Cilev

[1]University St. Kliment Ohridski-Faculty of Veterinary Medicine, str. Prilepska bb, p.box. 150, 7 000 Bitola, Republic of Macedonia.
*Corresponding author: Prof. dr. sc. Goce Cilev, Ph.D; E-mail: goce_cilev@yahoo.com
Invited paper

Abstract: To examine the possibilities of the maize's substitution as an energetic nutrient with by-products obtained by manufacturing tomatoes, peppers and grapes in the nutrition of different categories of swine on the production results and health condition where are carried out experiment in the nutrition of weaned piglets.The researches were conducted in the producing conditions at pigs farm ZZ "Edinstvo" in the village of Chelopek near by the town of Tetovo, R. Macedonia where were organized experiment in a group controlled system. The experiment is carried out on piglets after weaning in a period of 28-30 days with an average body weight of 8.66±0.21 kg (K); 8.79±0.20 kg (O-I) and 8.84±0.19 kg (O-II). For the experiment used mongrels of Swedish and Dutch races with equalized genetical potential. Each group in experiment consist of equal number of males and females. The experiment of the weaned piglets is carried out on 72 piglets divided into 3 groups with each group having 12 piglets of different sex. The experiment lasted for 42 days in 2 phases with 21 days each phase. In the experiment were used 2 mixtures for weaned piglets from 1–21 days and the whole mixture for piglets in breeding in a period of 21–42 days of the experiment. The piglets from the control group (K) from experiment were fed with mixture without a share from the examined by-products, whereas the sample groups were fed with food with substitution of the maize with different qualities of the above mentioned by-products. In the piglets feeding mixture – 3% (O-I) i.e.6% (O-II) from the examined by-products. Average Body Weight (ABW) in the control group (K) and experimental groups (O-I and O-II) in the end of the growing period (42 day) were 26.98 kg, 27.37 kg and 26.02 kg, respectively. Average Daily Gain (ADG) and Average Feed Conversion (AFC) in the control group (K) and experimental groups (O-I and O-II) for all period (1-42 days) were (436 g *vs* 1.51 kg-K), (445 g *vs* 1.50 kg-O-I) and (409 g *vs* 1.47 kg-O-II). No significant difference was observed in all three groups ($p>0.05$). Maize's partial substitution with a by-products obtained by manufacturing tomatoes, peppers and grapes especially in the amount of 3% does

not have any negative effects in the production results on a weaned piglets. In overall, the applied treatments don't have negative influence in the production results and health condition of different categories of swine which enables real possibilities for maize's semisubstitution with a by-products obtained by manufacturing tomatoes, peppers and grapes.

Key words: nutrition, weaned piglets, by-products obtained by manufacturing, tomatoes, peppers and grapes, production results

Introduction

The intensive animal production is very demanding towards the organism of the domestic animal, as well as the animal food industry. The goal of the intensive pig production is high growth with minimal food consumption in shortest time giving animal products without harmful substances at the same time taking care of the animal's health. Because of the differences in nutrition, husbandary, accomodation and pig breeds in our conditions of production we only review the literature that is a result of extensive research done on weaned piglets production (*Kovčin and Pejić, 1988; Waaijenberg, 1987; Puača, 1970; Collier and Hardy, 1986; Rotar and Salobir, 1987; Inborr, 1989; 1990; Inborr, 1989a; Officer, 1992; Cowan, 1995; Easter, 1995; Graham, 1996; Partridge, 1997; Grujić, 1998; Dabetić, 1999)*.

Breeding animals has developed as means of providing enough quantities of food from animal origin that is biologically valuable. Achieving it, there are two main problems that need to be solved: producing large quantities of industrial feeds for animals and dealing with the shortage of certain feeds. One of the prerequisites for producing industrial feeds for animals is providing each feed, especially those high in energy which constitute above 40% of the total mixture. In Macedonia 80% of the energetic feeds can be obtained from the production by the domestic industry, the other 20% are imported. Unfavorable climatic conditions further complicate the situation with energetic feed production, especially maize. The difficulties apply to the export of feeds too. So beside the hampered foreign trade, the production of feeds for animals and the animal breeding in general depends on imported feeds. That justifies the striving for solutions for rational using of the energetic feeds of domestic origin, and substituting them, e.g. with by-products from the food industry. The non-hazardous disposal of by-products obtained from the food industry during the manufacturing of foods from plant origin into human consumption foods further actualizes the situation.

The research to date has noted and established in practice a great number of by-products. Especially, there should be mentioned the by-products obtained from the wheat industry (wheat flour, wheat bran), cooking oil industry (pellets), sugar industry (dry slices of sugar beet). In Macedonia there are other by-products that

can be obtained from the agrocomplexes. The unconventional by-products that receive most attention are those obtained from tomatos, peppers and grapes. The waste from the manufacturing of the tomato, peppers and grapes is 5-10, 25-30 and 20-25% respectively. Most of the published work on the nutritional value of the above mentioned by-products bring out the energy value and relatively good protein value *(Sinovec and Ševković, 1995; Radovanović and Rajić, 1990; Stojanović et al., 1989; Todorov, 1995; Šokarovski and Cilev, 1999*). Biological experiments proved their nutritive value and efficiency in nutrition of ruminants (*Smilevski et al., 1973, 1975; Damjanovska et al., 1988; Šokarovski et al., 1981; Bogdanov, 1980*). Since the demand for pork and poultry is increasing, it is necessary to examine the effect of this by-products in the nutrition in non-ruminants, especially that they compete for food with humans.

That's why it is scientifically justified and practically usefull to examine the effects of the maize substitution with by-products obtain from the manufacturing of the tomatos, peppers and grapes, on the production results and health of the weaned piglets.

Materials and Methods

The objective was to examine the possibility of maize substitution with by-products obtained by manufacturing tomatoes, peppers and grapes in the nutrition of weaned piglets. The chemical composition of by-products obtained during processing of tomatoes, peppers and grapes used in the experiment is shown in Table 1.

Table 1. The chemical composition of the tested by-products and corn, [%]

Chemical composition	By-products			Corn
	Grapes	Peppers	Tomatoes	
Moisture	8.40	8.61	8.18	13.00
Ash	4.36	6.15	3.38	1.20
Proteini	12.66	18.77	21.15	8.00
Fat	10.60	8.18	13.20	4.00
Fibre	39.16	37.78	39.31	2.10
NEM	24.82	20.51	14.78	71.70
Calcium	0.64	0.56	0.41	0.02
Phosphorus	0.41	0.82	0.36	0.30
ME, MJ/kg	8.99	8.50	8.61	13.97
Lysine	0.33	0.29	0.31	0.20
Methionine+cystine	0.13	0.15	0.11	0.26
Threonine	0.18	0.10	0.02	0.10
Tryptophane	0.35	0.25	0.22	0.40

The table notes that, tested products contain significantly higher amounts of protein and fat compared to corn, with slightly less favorable protein has the amino acid composition. On the other hand, due to the extremely high content of fiber and very low carbohydrate content, was much poorer source of energy than corn.

An experiment by group control system was carried out and the effects on the productive results and health conditions of the piglets were measured at the producing condition on the pig farm ZZ "Edinstvo", village Chelopek, Tetovo region, R. Macedonia. The experiment was carried out on weaning piglets about 28-30 days old and average weight of 8.66-8.84 kg. Mongrels were used for the experiment from Swedish and Dutch races with equalized genetic potential. Each group consisted of equal number of males and females.

The experiment of the weaning piglets was carried out on 72 piglets divided into 3 groups, each group consisting of 12 piglets of equal sex proportions. The experiment lasted for 42 days in 2 phases each lasting for 21 days. The weight of the piglets and the amount of consumed feed were measured on a regular basis during the experiment. The weaned piglets were fed with suitable mixture with standard raw and chemical composition (table 2). Two mixtures were used during the experiment that completely satisfied the needs of the piglets (*AEC, 1993; NRC, 1998; Regulation 2000*) mixture for weaning piglets for 1-21 days, and mixture for breeding piglets for 21-42 days experimental period.

Table 2. Composition of mixture for the nutrition of weaning piglets, [%]

Feeds	to 15 kg				from 15-25 kg		
	K	O-I	O-II		K	O-I	O-II
Maize	61.70	58.70	55.70		65.30	62.30	59.30
By product of tomatoes	-	1.00	2.00		-	1.00	2.00
By product of peppers	-	1.00	2.00		-	1.00	2.00
By product of grapes	-	1.00	2.00		-	1.00	2.00
Soya bean meal	22.00	22.00	22.00		21.00	21.00	21.00
Sunflower meal	5.00	5.00	5.00		5.00	5.00	5.00
Fish meal	6.00	6.00	6.00		3.00	3.00	3.00
Soya oil	3.00	3.00	3.00		3.00	3.00	3.00
Limestone	0.50	0.50	0.50		0.50	0.50	0.50
DCaP	1.00	1.00	1.00		1.30	1.30	1.30
Salt	0.20	0.20	0.20		0.30	0.30	0.30
Methionine	0.05	0.05	0.05		0.05	0.05	0.05
Lysine	0.05	0.05	0.05		0.05	0.05	0.05
Premix	0.50	0.50	0.50		0.50	0.50	0.50

The main aim of the research was to determine how the maize substitution with by-products, obtained by manufacturing tomatoes, peppers and grapes in the nutrition of weaning piglets, effects the production results and the piglet's health condition

and to determine weather it is practical to use mixtures with by-products in the nutrition of piglets. Minimal corrections were made in the mixtures. The experimental piglets from the experimental control group were fed with mixtures not containing any of the above mentioned by-products, whereas the pigs in the experimental groups were fed only with mixtures where maize was substituted with different quantities of the specified by-products. The mixuters included 3 and 6% from the examined by-products in the mixtures for piglets nutrition.

Samples from the feed for analysis were taken at equal time intervals of 30 days during the experiment. *Regulation (1987)* for the methods of sampling and methods of physical, chemical and microbiological analysis of animal feeds was used.

Control weighing of the experimental animals was done using technical scale that has 10^{-2}kg accurate. Based on those results, the mean weight of animals was calculated. From the difference in the measurings the total weight gain was calculated, while the daily weight gain was calculated based on individual phases of the experiment, as well as the total experiment.

During the experiment the total mixture that was given to particular group of animals was weighted. In the end, based on the sum of the daily mixture that were used, the total expenditure of food was calculated for each phase and the whole experiment. The results on consumed feed and the weight gain of the pigs, the conversion of food was calculated for each phase and the whole experiment.

Beside taking preventive measures, all of the piglets were under veterinary control and every change in medical condition was examined and noted. Daily examination comprised group and individual observations.

Results and Discussion

The chemical content of the mixtures for the weaned piglets in the experiment is shown in Table 3. It can be seen that substituting the maize with by-products from the manufacturing of tomatoes, peppers and grapes insignificantly increases the content of protein, fat and fiber and energy, while the amino acids content remains the same.

The rations and amount of the feeds in the mixture for the experimental piglets were composed using conventional methods used in the practice. The results on the chemical content of the feeds used for the growing piglets in the control group showed that the food was meeting the technological standards and regulations (*Regulation, 2000*), and so the content of all the nutrients in the mixtures corresponded to the needs of the different categories of pigs in the different phases of the experiment (*NRC, 1998; AEC, 1993*). The chemical analysis

confirmed the balance in the content of different amino acids (lysine, methionine, treonine and tryptophane) in the mixture for the animals in the control group.

Table 3. Chemical composition of mixture for the nutrition of weaning piglets, [%]

Chemical composition	to 15 kg				from 15-25 kg		
	K	O-I	O-II		K	O-I	O-II
Moisture	11.32	11.17	11.04		11.45	11.31	11.18
Ash	5.06	5.17	5.27		4.90	5.00	5.11
Proteini	20.18	20.47	20.75		18.17	18.45	18.74
Fat	6.15	6.35	6.55		6.09	6.29	6.49
Fibre	3.80	4.90	6.00		3.80	4.90	6.00
NEM	53.49	51.94	50.39		55.59	54.05	52.48
Calcium	0.98	0.99	1.01		0.85	0.86	0.88
Phosphorus	0.74	0.75	0.75		0.70	0.70	0.71
ME, MJ/kg	14.39	14.43	14.48		14.32	14.37	14.42
Lysine	1.18	1.18	1.19		1.00	1.01	1.01
Methionine+cystine	0.69	0.69	0.69		0.62	0.61	0.61
Threonine	0.28	0.28	0.28		0.26	0.26	0.26
Tryptophane	0.45	0.44	0.44		0.43	0.43	0.43

The chemical content, including the amino acids, of the mixtures for the piglets in the experimental groups that had semisubstitution of the maize with by-products obtained from the manufacturing of tomatos, peppers, grapes, didn't differ significalnty from the mixtures for the control groups.

Based on the above, it can be concluded that the chemical content of the used total mixtures for all of the groups of experimental piglets was meeting the needs and corresponded to the demands that were made when the experiment was established.

The piglets in every group had uniform body structure, well formed bone and muscle tissue, vivid temper and were in good condition. The hair, skin and visible mucous membranes had no changes. Apetite was good, and the stool was normal. The ability for active movement and coordination of movement were well-balanced and the muscle tone was normal. During the experiment the health of the piglets was not affected and no disease or mortality was noted.

At the beggining, the piglets in all of the experimental groups had uniform body weight, and the differences between groups were not statistically significant (Table 4). At the middle of the experiment the experimental groups had slightly lower body weight compared to the control group, and the piglets in the group O-II had the lowest body weight. At the end of the experiment the piglets from all of the groups had almost the same body weight, and the piglets in group O-I had slightly higher body weight compared to the control group. The differences between the

groups were not statistically significant (p>0.05). Based on this results it can be already concluded that the used treatmets didn't affect the body weight.

Table 4. Body weight of weaning piglets in the experiment, [kg]

		Measures of variation					
Groups	**n**	**x**	±	**Sx**	**Sd**	**Cv**	**Iv**
1 day							
K		**8.66**		0.21	1.05	12.16	7.00-11.00
O-I		**8.79**		0.20	0.99	11.36	7.00-10.60
O-II		**8.84**		0.19	0.94	10.58	7.20-10.50
21 day							
K		**15.04**		0.86	2.74	19.38	10.30-17.80
O-I		**14.80**		0.79	2.51	17.96	8.70-16.70
O-II		**14.01**		0.80	2.52	19.14	9.30-16.30
42 day							
K		**26.98**		0.80	3.92	14.53	17.00-34.00
O-I		**27.37**		0.72	3.51	12.83	18.50-34.00
O-II		**26.02**		0.87	4.26	16.39	13.00-31.00

The average daily gain in mass in each group of the experiment had differences between the groups and in different phase (Table 5). The highest daily gain in weight in the first phase of the experiment was achieved by the control group, the lowest by the O-II group, the results being statistically significant (p<0.05). In the second phase of the experiment, the piglets of the two experimental groups increased their weight gain, but the results were not statistically significant (p>0.05).

Table 5. Gain of weaning piglets in the experiment, [kg/day]

		Measures of variation					
Groups	**n**	**x**	±	**Sx**	**Sd**	**Cv**	**Iv**
1-21 day							
K		**0.304**	[a]	0.01	0.01	11.41	0.205-0.422
O-I		**0.286**		0.02	0.05	12.28	0.185-0.410
O-II		**0.246**	[b]	0.03	0.08	15.35	0.185-0.380
21-42 day							
K		**0.568**		0.02	0.07	15.03	0.411-0.713
O-I		**0.598**		0.02	0.06	12.80	0.393-0.711
O-II		**0.572**		0.02	0.09	16.71	0.456-0.723
1-42 day							
K		**0.436**		0.01	0.06	13.32	0.337-0.504
O-I		**0.445**		0.02	0.06	14.71	0.309-0.564
O-II		**0.409**		0.03	0.07	15.33	0.382-.0.521

[a, b] p<0.05

The weight gain in the whole experiment was slightly higher for the O-I group, and slightly lower for the O-II group, when compared to the control group. Although the numbers show differences, the statistical anlysis concludes that the differences between groups in weight gain were not statistically significant ($p>0.05$).

Although the body weight is a good indicator for the quality of the nutrition, the daily gain is considered more accurate (Figure 1). From the data analysis it can be concluded that in both phases, the piglets in the control group that were fed with mixtures with standard raw material had an average daily gain of 0.436 kg. It is thought that feeding standard mixtures gives staisfying results on the production, and the pigs have a daily gain of 373 g *(Kovčin and Pejić, 1988*) to 383 g (*Waaijenberg, 1987*), and 0.340-0.387 kg *Dabetić (1999),* but in practice it has been measured (*Puača, 1970; Grujić, 1998*) even higher daily gains (0.415-0.430 kg).

The piglets in the both experimental groups had a lower weight gain compared to the control group, and the differences for the O-II group were statistically significant ($p<0.05$). In the second phase of the experiment, the piglets in both experimental groups had slightly higher weight gain, but the differences were not statistically significant ($p>0.05$). Talking about the whole experiment compared to the control group, the piglets from the group O-I had slightly higher, and the piglets from the group O-II slightly lower results in weight gain, and the analyis shows that the differences were not statistically significant ($p>0.05$).

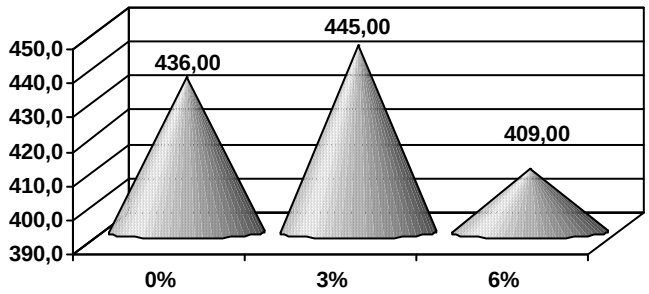

Figure 1. Daily gain [g]

The average daily consumation of feed is shown in Table 6. The piglets of the control group consumed the same amount as would in practice, and the same applied to the piglets in the first experimental group O-I. Increasing the percentage of the by products in the mixture had negative effect on consumation of feed by the piglets in the group O-II, both in different phases, and the whole experiment.

Table 6. Consumption of feed in the experiment, [kg/day]

	Groups		
Phase of experiment	**K**	**O-I**	**O-II**
	Weaning piglets		
1-21 day	**0.452**	**0.452**	**0.382**
Index	100.00	99.78	84.51
21-42 day	**0.865**	**0.890**	**0.820**
Index	100.00	102.09	94.60
1-42 day	**0.658**	**0.671**	**0.601**
Index	100.00	101.98	91.34

The apetite is a first indicator of the health of the animal and the quality of nutrition. The average daily consumation of feed varied between groups, especially between the experimental groups. The piglets in the control group that were fed with mixtures of standard composition of raw materials consumed the same amount that is standard for the pigs in practice, from 0.626 kg (*Waaijenberg, 1987*), 0.649-0.665 kg (*Dabetić, 1999*) to 0.760 kg (*Kovčin and Pejić, 1988*), and 0.790 (*Puača, 1970*), but some noted (*Grujić, 1998*) much higher values (0.941 kg) (Figure 2).

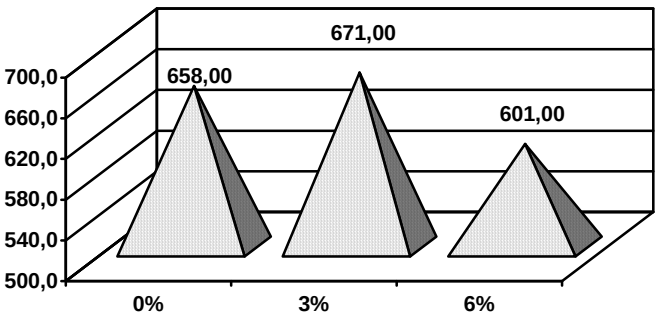

Figure 2. Comsumption of feed, [kg]

The piglets from the experimental groups consumed different amount of food, and the increase of the by products in the mixture decreased the consumation of food that applied to both phases, and the whole experiment.

Food conversion as well as the interaction of the growth and consumation of food, was different between the groups and in different phases, and between the experiments (Table 7). The pigs in both of the experimental groups had lower conversion in the first phase and higher conversion in the second phase of the

experiment. For the whole experiment the experimental groups had conversion that was lower by 1.97 and 2.64 respectively.

Table 7. Conversion of feed in the experiment, [kg]

	Groups		
Phase of experiment	**K**	**O-I**	**O-II**
	Weaning piglets		
1-21 day	1.49	1.58	1.55
Index	100.00	106.04	104.03
21-42 day	1.52	1.49	1.43
Index	100.00	98.03	94.08
1-42 day	1.51	1.50	1.47
Index	100.00	99.34	97.36

Food conversion, as an interaction of weight gain and feed consumation, is a resultant that at the end is the best indicator of the profitability of the production, which means the quality of nutrition and its ability to meet the specific and high needs for growing young animals. The piglets in the control group that were fed with mixtures of standard composition of raw materials achieved a conversion that is standard for the pig industry: from 1.63-1.81 kg (*Waaijenberg, 1987; Puača, 1970*), and 1.69-1.95 kg (*Dabetić, 1999*), as much as 2.05-2.18 kg (*Kovčin and Pejić, 1988; Grujić, 1998*). The growing piglets of the two experimental groups had a lower conversion in the first phase of the experiment, while the conversion in the second phase was better. Talking about the whole experiment, the experimental groups had almost identical food conversion (Figure 3).

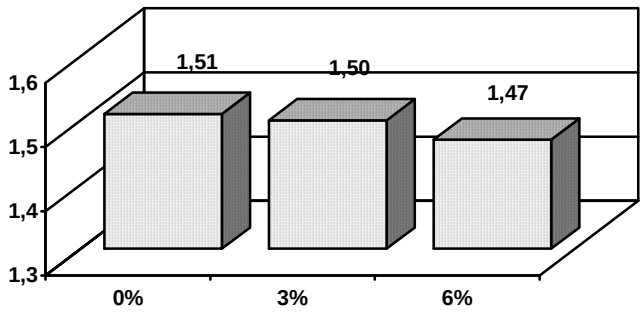

Figure 3. Conversion of feed, [kg]

Summing the results of the whole experiment, it can be stated that the semisubstitution of maize with by products obtained from the manufacturing of tomatoes, peppers and grapes, especially of 3%, has no negative effects on the production results of the weaning and growing piglets.

Conclusion

Based on the results from the examination of the possibilities for substitution of maize, as energetic feed, with by products obtained from the manufacturing of tomatoes, peppers and grapes in the nutrition of weaning and growing piglets over the production results and health it can be concluded that:

- Semisubstitution of maize with by products obtained from the manufacturing of tomatoes, peppers an grapes, especially of 3%, has no negative effects over the production results of the weaning and growing piglets.
- As a whole, the applied treatments do not negatively affect the production results and the health condition of the different categories of pigs, which advocates the semisubstitution of the maize with by products obtained from the manufacturing of the tomatoes, peppers and grapes.

Ispitivanje efikasnosti delimicne zamene kukuruza sa nusproizvodima dobijenim pri preradi povrca i voca u smjesama za odbijenu prasad

G. Cilev

Rezime

U cilju ispitivanja mogućnosti supstitucije kukuruza, kao energetskog hraniva, nus proizvodima dobijenim pri preradi paradajza, paprika i grožđa u ishrani razlicitih kategorija svinja na proizvodne rezultate i zdravstveno stanje izveden je ogled ishrane odbijene prasadi.

Istraživanja su izvedena u proizvodnim uslovima na svinjarskoj farmi ZZ “Edinstvo“ s. Čelopek iz Tetovskog, R. Makedonija gde je organizovan ogled po grupno-kontrolnom sistemu. Ogled je izveden na prasadima nakon odbijanja u dobi od oko 28-30 dana i prosečne telesne mase 8.66±0.21 kg (K); 8.79±0.20 kg (O-I) i 8.84±0.19 kg (O-II). Za ogled su korišćeni melezi švedskog i holandskog landrasa ujednačenog genetskog potencijala. Svaka grupa u ogledu se sastojala od podjednakog broja muških i ženskih životinja. Ogled na prasadima u odgoju je

izveden na ukupno 72 grla podeljenih u 3 grupe, a svaka grupa se sastojala od po 12 grla različitog pola. Ogled je trajao ukupno 42 dana i podeljen je na dve faze po 21 dana. U ogledu su korišćene dve smese i to: potpuna smesa za odbijenu prasad od 1-21. dana i potpuna smesa za prasad u odgoju od 21-42. dana ogleda. Grla kontrolne grupe (K) hranjena su smesama bez učešća ispitivanih nusproizvoda, dok su ogledne grupe dobijale hranu u kojoj je izvršena supstitucija kukuruza različitim količinama navedenih nusproizvoda: u smesama za hranidbu prasadi u odgoju 3% (O-I), odnosno 6% (O-II) ispitivanih nusproizvoda. Srednje telesne tezine u kontrolnoj grupi (K) i eksperimentalnim grupama (O-I i O-II) na kraju tovnog perioda (42 dan) su bile:
26.98 kg, 27.37 kg i 26.02 kg, respektivno. Srednji dnevni prirast i srednja dnevna konzumacija u kontrolnoj grupi (K) i eksperimentalnim grupama (O-I i O-II) na kraju celog tovnog perioda (1-42 dan) su bili (436 g *vs* 1.51 kg-K), (445 g *vs* 1.50 kg-O-I) i (409 g *vs* 1.47 kg-O-II). Nisu zabeležene statistički značajne razlike kod sve tri grupe ($p>0.05$).

Delimična supstitucija kukuruza nusproizvodima dobijenim u preradi paradajza, paprike i grožđa, posebno u količini od 3%, nema negativne efekte na proizvodne rezultate prasadi u odgoju. U celini, primenjeni tretmani ne utiču negativno na proizvodne rezultate i zdravstveno stanje razlicitih kategorija svinja što pruža realnu mogućnost delimične suptitucije kukuruza nusproizvodima dobijenim pri preradu paradajza, paprike i grožđa.

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POSSIBILITIES FOR THE PRODUCTION OF MEAT AND MEAT PRODUCTS WITH IMPROVED NUTRITIONAL AND FUNCTIONAL VALUE

D. Živković[1], S. Stajić[1], N. Stanišić[2]

[1] University of Belgrade, Faculty of Agriculture, 11080 Belgrade-Zemun, Republic of Serbia
[2] Institute for Animal Husbandry, Autoput 16, 11080 Belgrade-Zemun, Republic of Serbia
Corresponding author: dule33@agrif.bg.ac.rs
Invited paper

Abstract: During the past decade more attention has been paid to improving nutrition by increasing the beneficial aspects of food and reducing or eliminating those negative ones. By adding functional components to animal feed or directly to meat products it is possible to enhance their nutritional characteristics and produce functional food. Our experiments includes the addition of extruded linseed seed and conjugated linoleic acid (CLA) to pig and chicken feed, while linseed oil was added to fermented sausages, as oil-in-water emulsion. Extruded linseed seed added to animal feed helps raise the content of the PUFA ω-3 group ($p<0.01$), in the amount of 363–342% in pig loin meat and 122–162% in chicken legs. The ratio of ω-6/ω-3 in the meat of pigs and chickens in the test groups was considerably improved ($p<0.01$), reaching values close to optimum in chicken meat (5.33 and 5.77). The addition of CLA to pig feed caused a statistically significant reduction in total PUFA and PUFA ω-3 in ham meat and a statistically significant increase in CLA and enhanced ω-6/ω-3 group ratio. The greatest enhancement in fatty acid composition can be achieved through direct addition of oil as oil-in-water emulsion: by adding 5% linseed oil the ω-6/ω-3 ratio is reduced to 1.79, and the effects of oil addition are predictable and can be calculated in advance. Based on the results obtained, it can be concluded that indirect methods are applicable in the production of fresh meat and direct methods in the production of meat products.

Key words: pork, chicken, fermented sausages, functional food, ω-3 fatty acids

Introduction

During the past decade more attention has been paid to improving nutrition by increasing the beneficial aspects of food and reducing or eliminating those negative ones. There has been increased interest in reducing potentially harmful or risky

characteristics of meat and meat products and in increasing their functional properties. Though the term "functional food" has not yet been defined precisely *(Roberfroid, 2000)*, such food must contain components with selective effects on one or more functions in the organism, and, in turn, these effects must be physiologically functional or even "healthy". In order for food to be functional, apart from primary (energy) and secondary (structural) effects, it must also contain tertiary ones *(Dentali, 2002)*. Tertiary effects imply functional effects of certain nutrients with physiological impact which also exhibit anticancerogenic, antimutagenic and antioxidative effects, as well as slow down the aging process.
From a chemical standpoint, meat is a complex foodstuff comprising a number of nutrients and multifunctional compounds. For the major part of the world's population meat is the key source of biologically valuable proteins, essential amino acids, vitamins and minerals *(Biesalski, 2005)*. It also contains a series of functional bioactive components: carnitine, carnosine, coenzyme Q10, choline, taurine, conjugated linoleic acid, anserine and glutathione *(Arihara, 2004)*. Peptides responsible for product flavour are formed through the processes of fermentation and endogenous proteolysis *(Živković et al., 2010; Živković et al., 2012)* and, in addition, bioactive oligopeptides with hypotensive activity, antioxidant and opioid effects can also be formed *(Saiga, 2003; Vercruysse, 2005)*. On the other hand, meat lipids do not have such good characteristics, but rather properties such as high fat and cholesterol content, high energy and atherogenic value *(Swapna et al., 2012)*, bad ratio of saturated (SFA), monounsaturated (MUFA) and polyunsaturated fatty acids (PUFA), low content of ω-3 PUFA, as well as a poor ratio of ω-6/ω-3 *(Delgado-Pando et al., 2010)*. Such lipid content can be related to obesity, atherosclerosis, diabetes and coronary diseases, as well as increased risk of some cancers *(Ovesen, 2004a; Ovesen, 2004b)*. In some products, such as fermented sausages, the negative effects of high energy value are particularly pronounced (fat content 40–50%), and a high sodium content *(Živković et al., 2009; Živković et al., 2011)*.

Adults are recommended by health specialists to take in up to 30% of their daily energy needs through lipids, of which not more than 10% through SFA, 6–10% through PUFA (5–8% through the ω-6 group; 1–2% through the ω-3 group), and 10–15% through MUFA *(WHO 2003)*. The recommended ω-6/ω-3 ratio is less than 4 *(Wood, 2004)*, and of PUFA/SFA higher than 0.4 *(COMA, 1984)*, as well as up to 300 mg of cholesterol a day *(Živković et al., 2002)*. Daily need for fatty acids in the ω-3 group is around 1.6 g for adults *(Food and Nutrition Board, Institute of Medicine, 2002)*.

A lot can be done to enhance the nutritional and functional properties of meat and meat products *(Zhang et al., 2010; Arihara, 2004)*, and we can divide such activities into direct and indirect ones *(Swapna et al., 2012)*. With indirect

activities, animals through their food receive components that cause changes in meat characteristics (including milk and eggs), which is a longer-lasting project and for the time being not fully controlled, whereas in direct activities, we add functional components directly into meat or meat products, which is relatively quick and simple.

Indirect activities aimed at increasing nutritional and functional value of meat

Nutrition is the easiest way to influence the composition and quality of muscle and fatty tissues, and in turn the nutritional and functional value of meat, especially in monogastric animals (pigs, poultry and fish) since their organism absorbs fatty acids in their intact form. Conversely, fatty acids taken in through food are altered in the digestive tract of ruminants due to biohydrogenation in the rumen.

In order to improve the fatty acid composition of meat, the authors propose the use of fish and plant oils in the animal diet *(Zhang et al., 2010)*. Recommended plant oils are those with a high content of ω-3 PUFA, such as linseed and rapeseed oil, which have a conditionally passive metabolic function. As for fatty acids with active metabolic functions, the authors cite various isomers of linoleic acid (C18:2).

For our first experiment, we used extruded linseed seed as the source of ω-3 PUFA and added it into pig and chicken feed. In our experiment with pigs, we used generation F1 porkers (Yorkshire x Swedish Landrace) x Pietren, weighing around 60 kg, which were given 4% of extruded linseed seed through their feed during the last 60 days of fattening. The porkers were slaughtered when they were 180 days old. The experiment was carried out at the pig farm of meat company "Union MZ", Petrovac. In our experiment with chickens, we used broilers of the Ross hybrid line who were given 6% of extruded linseed seed through their feed during the last 22 days of fattening. The chickens were 45 days old at the moment of slaughter. The experiment was carried out at the chicken farm of meat company "Union MZ", Svilajnac.

Table 1 shows data pertaining to the characteristics of fatty acids from the meat of pork loin and chicken legs of animals fed by a standard mixture of feed without the addition of extruded linseed seed, linseed oil and the values of basic nutritional recommendations. It is evident that due to its characteristics, pork cannot be considered a foodstuff that is a source of the ω-3 PUFA. In terms of perhaps the most important parameter, ω-6/ω-3 ratio, its nutritional status is quite poor. Chicken meat has a relatively favourable PUFA/SFA ratio, however, in terms of other characteristics, it is beyond the recommended nutritional framework.

Table 1. Composition (% of total fatty acids) of pig and chicken meat, and linseed oil

Fatty acid composition (profile)	Pork loin (average male and female)	Chicken leg (average male and female)	Linseed oil (Delgado-Pando 2010)	Health recommendation (COMA 1984; Food and Nutrition Board, Institute of Medicine, 2002; Wood 2004;)
SFA	40.52%	30.41%	9.94 %	-
MUFA	50.52%	41.80%	21.74%	-
PUFA	8.33%	27.21%	68.31%	-
PUFA/SFA	0.2	0.90	6.87	> 0.4
Total ω-6	8.13%	25.27%	15.23%	-
Total ω-3	0.20%	1.96%	53.08%	1.6 g per day
ω-6/ω-3	42.78	13.05	0.29	< 4

Owing to the addition of linseed, ω-3 PUFA content rose by 363% in the meat of female porkers and by 342% in the meat of male porkers (Figure 1). Although this increase was multifold and statistically significant ($p<0.01$), it, nevertheless, was not of such scope to make a crucial contribution to the increase in nutritional value of pork. Due to the higher ω-3 PUFA content, the ω-6/ω-3 ratio changed considerably ($p<0.01$) (Figure 2); thus, although it did not reach the recommended value, this effect can still be considered very useful as it proves that significant corrections are possible and that the ω-6/ω-3 ratio can be corrected to optimum values.

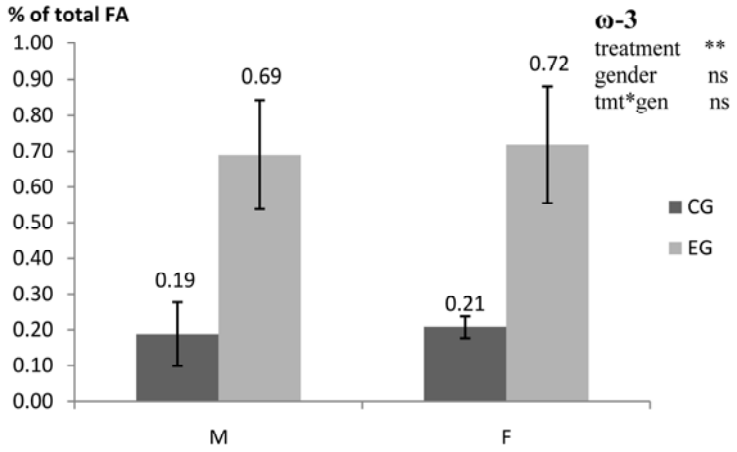

Figure 1: PUFA ω-3 content in loin meat of pigs in control and test groups

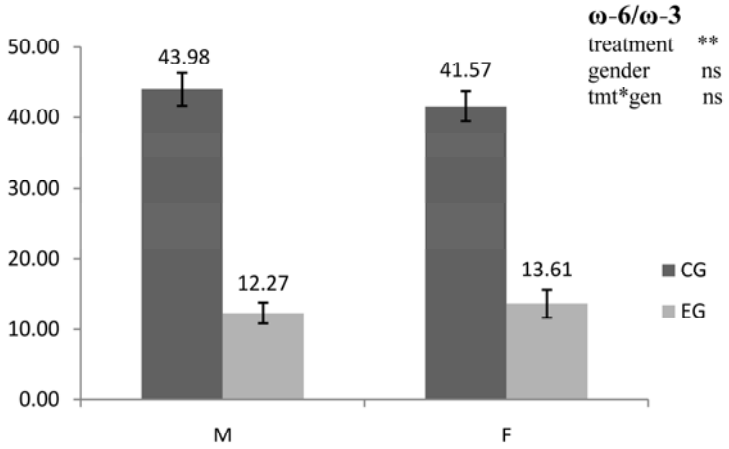

Figure 2: ω-6/ω-3 group ration in loin meat of pigs in control and test groups

The data pertaining to the effects of adding linseed seed to chicken feed are shown in Figures 3 and 4. The addition of extruded linseed seed to broiler feed resulted in a lower increase of ω-3 PUFA than in with pigs. The content of the ω-3 PUFA rose by 122% in the meat of males and by 162% in the meat of females. This increase is statistically significant ($p<0.01$). In order to meet the daily need for ω-3 fatty acids, around 400 g of such meat should be consumed. The ω-6/ω-3 ratio was considerably improved ($p<0.01$) and its values in the meat of chickens fed with the addition of extruded linseed seed were close to optimum. The results obtained are fully compatible with those of *(Lopez-Ferrer et al., 2001)* who determined that with the addition of fish oil, the ω-3 fatty acid content in chicken meat can increase multifold.

Conjugated linoleic acid (CLA) as an active metabolic modifier affects the composition of meat fatty acids, and can also affect the outcome of fattening, carcass conformation and most notably the width of subcutaneous fat, and the technological, sensory and nutritional quality of meat. In addition, the CLA has been confirmed to have a functional effect, i.e. anticancer and anti-atherogenic effect, to positively affect the immune system, metabolism of lipids, diabetes and obesity *(Park, 2009)*.

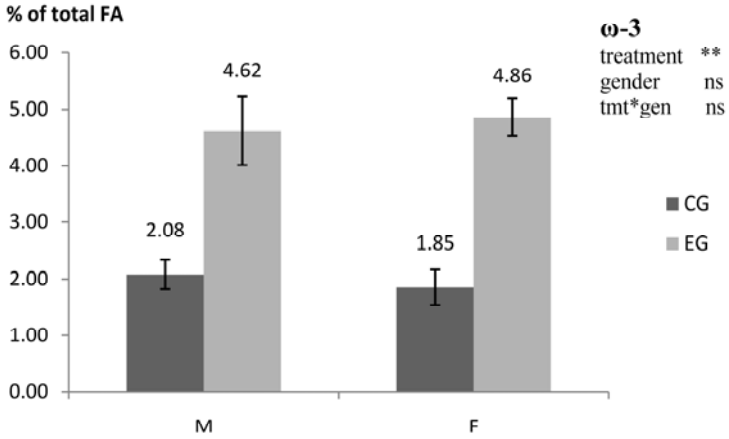

Figure 3: PUFA ω-3 content in chicken leg meat of control and test group of chickens

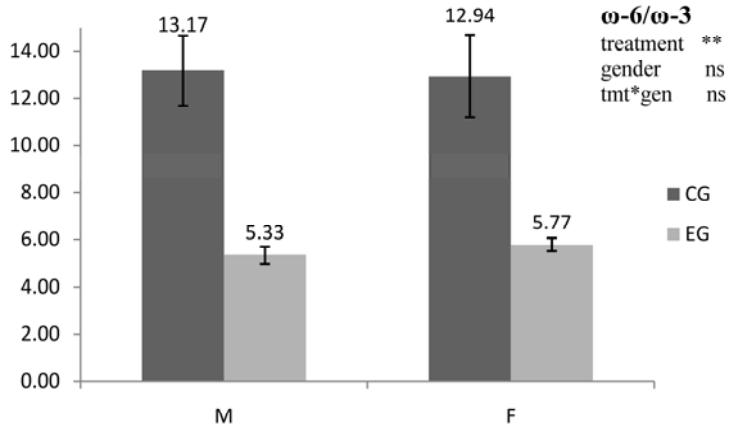

Figure 4: ω-6/ω-3 group ratio in chicken leg meat of control and test group of chickens

In the second experiment, we used the CLA in the form of Lutalin™ (BASF Corporation, Germany), that contains, as an active component, 60% CLA, (30% cis-9, trans-11 i 30% trans-10, cis-12 isomers in the 1:1 ratio). In the experiment, we used Swedish Landrace porkers, and the CLA oil was added to their feed during the last 45 days of fattening (from day 130 to day 175), therefore each animal in the test group was given 15 g of CLA oil on average a day, i.e. a total of 675 g of CLA oil during the entire experiment.

Table 2. Effects of CLA addition on fatty acid composition of *M. gluteus medius*

%	Male fatteners			Female fatteners		
	Control	Test group	p	Control	Test group	p
CLA	nd[6]	0,27 ± 0,02	*** (0,001)	nd	0,29 ± 0,03	*** (0,001)
SFA	40,24 ± 2,84	42,51 ± 3,75	*** (0,001)	40,33 ± 2,95	43,88 ± 2,86	*** (0,001)
UFA	59,77 ± 1,84	57,48 ± 2,75	*** (0,002)	59,68 ± 3,95	56,11 ± 2,55	*** (0,001)
MUFA	51,67 ± 2,42	50,81 ± 2,01	* (0,029)	50,15 ± 4,65	47,67 ± 2,62	*** (0,001)
PUFA	8,11 ± 0,88	6,68 ± 1,13	* (0,043)	9,52 ± 1,70	8,45 ± 1,57	ns (0,092)
ω-6	7, 67 ± 1,08	6,00 ± 1,12	* (0,020)	9,00 ± 0,69	7,67 ± 1,57	* (0,032)
ω-3	0,44 ± 0,12	0,42 ± 0,21	ns (0,852)	0,52 ± 0,11	0,50 ± 0,15	ns (0,782)
ω-6/ω-3	17,45 ± 2,51	14,29 ± 1,34	** (0,008)	17,32 ± 1,91	15,65 ± 2,24	* (0,015)

The addition of the CLA affected to a considerable extent the change in the composition of fatty acids of *M. gluteus medius* (Table 2). This change was reflected in a significant increase in the share of total saturated fatty acids (SFA) and a reduction in the share of total unsaturated (UFA) and monounsaturated fatty acids (MUFA), whereas the percentage of total PUFA was quite stable. The nutrition of pigs with the CLA had the largest effect on the composition of fatty acids in the ham meat of female porkers. The sums of ω-3 fatty acids did not differ considerably between the control and test groups of porkers of both sexes, though it was determined that their ratio (ω-6/ω-3) was considerably lower in pigs that were fed with the CLA. These data correspond fully to the results obtained by *(Joo et al., 2005; Pastorelli et al., 2005)*. From a nutritional and functional point of view, there are two different groups of effects. A reduction in the total content of PUFA, along with a decline in the ω-3 group content, both statistically insignificant, should not be a nutritional recommendation, whereas a considerably more favourable ω-6/ω-3 ratio and increased content of the CLA in meat are most certainly positive effects.

Direct activities aimed at increasing nutritional and functional values of meat

These activities are based on the change in the profile of fatty acids by way of adding functional components directly into the product. In terms of enhancing the lipid content, fish and plant oils (linseed, rapeseed and hemp oil) with a high ω-3 fatty acids content are most used to substitute one part of fatty tissue. Oil addition is a technologically simple operation when it comes to emulsified products, but far more complex with fermented sausages. Oil is usually added as pre-emulsified (oil-in-water emulsion) with soy protein isolate (SPI), alginate and Na-caseinate and encapsulated. Bearing in mind that fat contributes to the colour, texture and flavour

of fermented sausages, the addition of these oils makes sense only if the sensory quality is either not altered or slightly changed, because otherwise the acceptability of a product would be reduced.

In the third experiment, we used linseed oil pre-emulsified with soy protein isolate in the 5:5:1 (water:oil:SPI) ratio. The oil was added in the mixture for dry fermented sausages in the amount of 5%, 7% and 9%.

The results shown in Figure 5 illustrate that through direct addition of oil with high ω-3 content the share of ω-3 fatty acids can be greatly increased, i.e. that with 5% of added linseed oil a functional fermented sausage-type of product can be produced through which the daily need for ω-3 fatty acids can be secured by consuming no more than 30 g of the product. The addition of linseed oil (Figure 6) also has a significant effect of the reduction of the ω-6/ω-3 ratio to ideal values. This way, the fatty acid composition of fermented sausages can be significantly improved to make them more nutritionally desirable. The effects of oil addition are predictable, i.e. it is possible to calculate the amount of oil that should be added to the product to achieve the desired fatty acid composition, and the only limitation as to the amount of oil are the sensory characteristics of the finished product (*Stajić et al., 2012*)

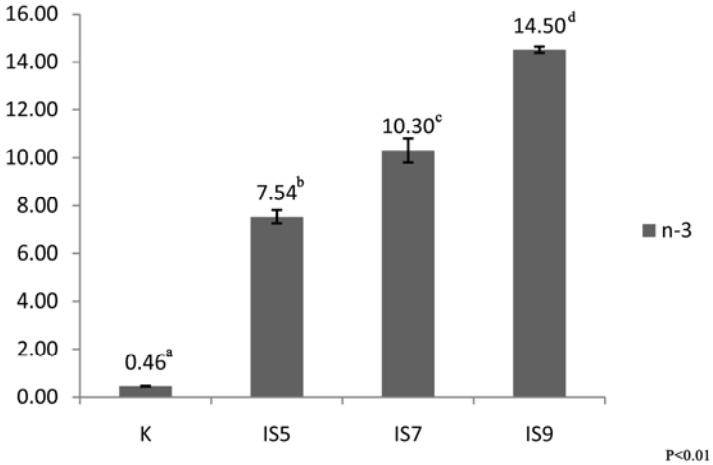

Figure 5: PUFA ω-3 content in dry fermented sausages (C–control; IS5–5% oil; IS7–7% oil; IS9–9% oil)

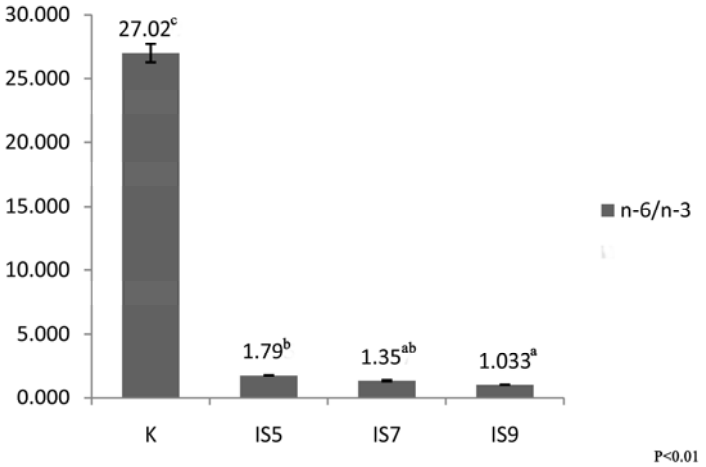

Figure 6: ω-6/ω-3 ratio in dry ferment sausages (C–control; IS5–5% oil; IS7–7% oil; IS9–9% oil)

Conclusion

Extruded linseed seed, with a high content of the ω-3 PUFA, when added to animal feed can considerably enhance the fatty acid composition of pork and chicken. The content of the total ω-3 PUFA can thus be increased multifold, and the otherwise poor ω-6/ω-3 ratio can be corrected to equal the optimum value. These effects are both statistically significant and large scale.

The addition of the CLA resulted in statistically significant reduction in the content of total PUFA and ω-3 PUFA, and at the same time in a statistically significant increase in the CLA and a better ω-6/ω-3 ratio. The achieved intensity of changes is smaller in scope than in the case of linseed seed, although it is difficult to draw a direct comparison between the effects.

The largest changes in the fatty acid composition can be achieved through direct addition of oil into products. The effects of oil addition are predictable and almost instantaneous therefore there are grounds to claim that indirect methods are applicable in meat production, and direct ones in meat products.

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Mogućnosti proizvodnje mesa i proizvoda od mesa sa povećanom nutritivnom i funkcionalnom vrednošću

D. Živković, S. Stajić, N. Stanišić

Rezime

Tokom poslednje decenije sve veća pažnja se poklanja unapređenju ishrane sa težnjom da se potencira sve što je u namirnici korisno, a umanji ili otkloni ono što je nutritivno ili zdravstveno negativno. Dodatkom funkcionalnih komponenti u hranu za životinje (indirektne metode) ili direktno u proizvode moguće je popraviti nutritivna svojstva mesa i proizvoda od mesa i na taj način proizvesti funkcionalnu hranu. U ovim ispitivanjima ekstrudirano seme lana i konjugovana linolna kiselina (CLA) dodavani su u hranu za svinje i piliće, a laneno ulje je dodavano u fermentisane kobasice kao emulzija ulja u vodi.

Ekstrudirano seme lana, sa visokim sadržajem PUFA ω-3 grupe, dodato u hranu za životinje, može značajno popraviti masnokiselinski sastav svinjskog i pilećeg mesa. Sadržaj ukupnih PUFA ω-3 grupe je na ovaj način moguće višestruko povećati, a inače loš odnos ω-6/ω-3 grupa može se korigovati do optimalnog. Dodatak ekstrudiranog semena lana u hranu za životinje utiče na povećanje sadržaja PUFA ω-3 grupe ($p<0.01$), koje iznosi 363-342% u mesu slabina svinja, a 122-162% u mesu bataka sa karabatacima pilića. U mesu svinja i pilića, hranjenih sa dodatkom ekstrudiranog semena lana, odnos ω-6/ω-3 se značajno popravio ($p<0.01$) a ove vrednosti su u mesu pilića bliske optimalnim (5.33 i 5.77). Dodatak CLA hrani za svinje uticao je na statistički značajno smanjenje ukupnih PUFA i PUFA ω-3 grupe u mesu buta, a značajno povećanje sadržaja CLA i poboljšanja odnosa ω-6/ω-3 grupa.

Direktnim dodavanjem ulja, kao emulzije ulja u vodi, moguće je ostvariti najveće izmene masnokiselinskog sastava, sa dodatkom 5% lanenog ulja, odnos ω-6/ω-3 smanjuje se do vrednosti 1.79, pri čemu su efekti dodavanja ulja predvidivi, i moguće ih je preračunati. Na osnovu dobijenih rezultata može se konstatovati da su indirektne metode primenjive u proizvodnji svežeg mesa, a direktne u proizvodima.

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CONJUGATED LINOLEIC ACID IN PIG NUTRITION: EFFECTS ON FATTENING PERFORMANCE AND CARCASS COMPOSITION

N. Stanišić[1], D. Živković[2], M. Petrović[2], N. Delić[1], G. Marinkov[1], Lj. Stojanović[1]

[1] Institute for Animal Husbandry, Autoput 16, 11080, Belgrade-Zemun, Republic of Serbia
[2] Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080, Belgrade-Zemun, Republic of Serbia
Corresponding author: nikola0135@yahoo.com
Invited paper

Abstract: In recent years a number of studies have been carried out related to the effects of feeding animals with conjugated linoleic acid (CLA). CLA is a mixture of geometric isomers of linoleic acid, which are determined by various positive effects such as the impact on improving the feed conversion and the impact on the reduction of body fat and increase of the share of muscle tissue in the body of animals. The reason for the great interest in this compound is its potential health effect on human population, such as anti-cancer effect, enhancement of the immune system, reduction of cardiovascular disease, etc. Feeding pigs with the addition of CLA is gaining increasing importance in recent years, because it affects the improvement of feed conversion, reduces thickness of subcutaneous fat tissue and increases meat yield. However, above mentioned positive effects were not confirmed in every trial where pigs were fed diets containing CLA, because there are many factors that affect its efficiency.

Key words: CLA, pig, fattening performance, carcass composition

Introduction

The requirements in terms of quality of pig carcass over time have changed. Fat has long been the most sought-after product, while the meat was secondary. Over time, the demand for pork fat decreased, and for meat increased (*Stanišić et al., 2013*). In the European Union, and in Serbia, today the pig price depends largely on the share of muscle tissue in the carcass. As a result, the effective genetic selection created the genotypes of pigs with high lean meat content and optimal performance, ie. minimum cost of production, which are reflected primarily in increased gain and improved feed conversion.

In recent years, science and expert efforts were focused on the reduction of fat tissue content in fattening animals, especially pigs (*Stanišić et al., 2011a*). As a result, particularly of the improvement of the genetic basis, modern high quality pig breeds and their crosses, as well as progress in the field of animal nutrition, fat thickness at the back part is reduced to less than 10 mm, and the content of the muscle tissue in the carcass often exceeds 60%. Pigs are now selected, to have an efficient feed conversion and to better convert the food into muscle tissue (*Schinckel, 2001*). However, as the fat is one of the most important edible products derived from slaughtered pigs and one of the main ingredients for the production of quality meat products (*Stanišić et al., 2011b, 2012a*), its too low content in the carcass may have a negative economic effect.

Factors influencing the fattening performance and carcass composition of pigs

The success of pig fattening depends on several factors, such as the choice of genotype, rearing, meal balance, composition and quality of raw materials in feed mixtures, method of feeding of pigs, zoohygienic requirements, health of pigs, etc. From an economic point of view, success of fattening is assessed through the fattening performance of pigs, which is a very important indicator of production, and it is indicated by the rate of growth (weight gain) and feed consumption per kilogram of gain (feed conversion-GF) (*Kralik et al., 2007*). With the increase of daily gain, the growth of pigs is faster, fattening shorter, feed conversion reduces and the number of production cycles increases, all of which has impact on reducing production costs.

The carcass composition of slaughter animals is under the influence of breed, sex, diet and housing conditions (*Wagner et al., 1999*), and these factors have also an effect on the composition and quality of meat obtained. In addition, the quality of meat is influenced by conditions during transport of animals, the time spent in the depot slaughter, stunning procedure and primary treatment and the conditions of cooling and storage (*Stanišić et al., 2012b*).

The study of factors affecting the fattening performance and carcass composition is very complex, because the effect of any of these factors is not independent and can not be viewed in isolation. Correlations and interactions between factors have not yet been fully studied, so it is difficult to isolate the impact of each factor (*Teodorović and Radović, 2004*). However, those who have the greatest influence on the characteristics of the pig carcass and meat, which are under the control of the pig breeder, are the genetic basis (breed), age, weight, sex and castration and feeding of pigs.

In recent years a number of studies have been carried out related to the effects of feeding animals with conjugated linoleic acid. One of the reasons for the great interest in this compound is its potential health effect on human population, such as anti-cancer effect, enhancement of the immune system and reduction of the

occurrence of cardiovascular disease (*Cook, 1999*). Some of the effects of pig diet containing CLA, which has been documented in scientific publications are: improved feed conversion, reduced thickness of subcutaneous fat tissue, increased marbling of meat, increased formness of fat tissue, improved meat colour and reduce susceptibility of meat to oxidation. However, above mentioned positive effects were not confirmed in every trial where pigs were fed diets containing CLA, because there are many factors that affect its efficiency.

Conjugated linoleic acid

Conjugated linoleic acid (CLA - Conjugated Linoleic Acid) is a mixture of geometric isomers of cisΔ^9, cisΔ^{12} – octadecadienoic (linoleic acid - 18:2 n-6) acid, for which many positive effects were identified, such as anticancer effect, improved feed conversion and reduction of the amount of fat and increase of the share of muscle tissue in the body of animals *(Pariza and Hargaves, 1985; Ha et al., 1987)*. The name "conjugated" comes from the fact that the double bonds in the molecule are separated by two carbon atoms connected with a single bond. According to the location of the double bond and the trans/cis combinations, there are 16 different forms of CLA isomers *(Pastoreli et al., 2005)*.

CLA occurs naturally through bacterial biohydrogenation of linoleic acid (18:2 n-6) in rumen of ruminants through activity of several types of micro-organisms (*Griinari et al., 2000*). Consequently, the food produced from ruminants, meat and milk, are the main sources of CLA in the human diet (*Chin et al., 1992*). Although there is a large number of isomers of CLA in the food, the most common are cisΔ^9, transΔ^{11} (cis-9, trans-11) and transΔ^{10}, cisΔ^{12} (trans-10, cis-12) (Figure 1).

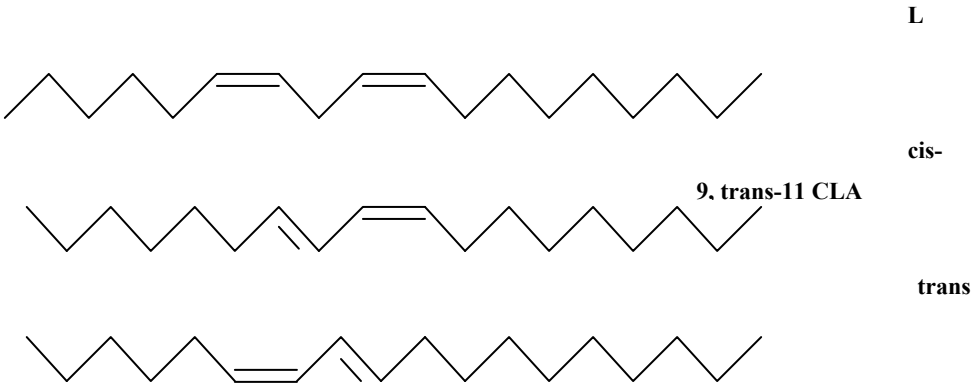

Figure 1. Structure of linoleic acid (A), cisΔ^9, transΔ^{11} – conjugated linoleic acid (B) and transΔ^{10}, cisΔ^{12} - conjugated linoleic acid (C)

Most of the research related to the CLA is focused on modifying the composition of the feed in order to increase the level of CLA in milk, meat and other products of animal origin. CLA can occur naturally, biosynthesis by ruminants or by chemical synthesis, in order to produce a variety of synthetic pharmaceuticals, as an active component of the mixture containing CLA isomers in various proportions.

The influence of CLA on fattening performances of pigs

There are different, and sometimes conflicting data on the activity of CLA on the growth of animals. These differences may be due to inclusion of CLA in various stages of fattening, because CLA acts differently in the first compared to the later stages of fattening and the finish. In addition, various studies have included a variety of species, and one of the issues that were included in researches was the stability of CLA that was used during the experiment.
Literature data on the impact of dietary CLA on pig production results vary considerably. *Dugan et al. (1997),* in a trial with 108 pigs (54 male and 54 female), average weight of 61.5 kg at the beginning of the experiment and the pre-slaughter weight of 106 kg, have determined the reduction in the amount of food consumed in pigs fed 2% CLA oil (by 5, 2% p=0.07) and improved GF (by 5.9 p=0.06), but similar ADG compared with pigs in the control group, fed with 2% sunflower oil, regardless of the sex of fatteners. A similar conclusion was reached two years later by *Ostrowska et al. (1999),* who investigated the effect of replacing soybean oil with different levels of CLA in the diet of fattening pigs females. Addition of CLA to the diet had no significant effect on performance of fattening pigs in the experiment of *O'Quinn et al. (1998), Ramsay et al. (2001), Martin et al. (2008), Corino et al. (2008)* and *Barnes et al. (2012). Wiegand et al. (2001)* have not found any change in ADG of male pigs fed 0.75% CLA, but stated that the relationship between average daily gain and feed intake increased significantly - the control group 330g/kg to 350g/kg the treated pigs. *Dunshee et al. (1998)* have found a slight increase in ADG, while *Cook et al. (1998),* examining the effect of addition of 4.8 and 9.5 g/kg CLA to pig diet during the last 84 days of fattening, have established the reduction of ADG in first 49 days of the experiment.

Thiel-Cooper et al. (2001) have found improved ADG and GF in pigs fed CLA with the addition of 26.3 to 114 kg of body weight. The above authors have investigated the effect of replacing corn oil with 0.12, 0.25, 0.5, and 1.0% CLA in the diet for male pigs, and they have found a positive correlation between the level of CLA in food and increase of ADG. *Szymczyk et al. (2005)* have reported that the addition of 0.4% CLA in the diet affects a significant increase in ADG of fattening pigs of both sexes. *Lauiridsen et al. (2005)* demonstrated improved GFR and ADG of pigs in which the sunflower oil in the feed was replaced with 0.3% CLA in the

final phase of the fattening (from 40 to 100 kg of weight) of female pigs. Replacement of soybean oil with CLA during the last 6 weeks of fattening of male pigs in the experiment of *Sun et al. (2004)* also had a significant impact on improving animal growth and feed conversion, and the authors have found a positive correlation between the length of the CLA diet and improved ADG. Regarding the influence of CLA on feed conversion, *Ostrowska et al. (1999), Thiel-Cooper et al. (2001)* and *Wiegand et al. (2002)* reported an increase, and *Dugan et al. (1997)* decrease of feed conversion ratio in pigs fed CLA. On the other hand, *Dunshee, et al. (1998)* and *Ramsay et al. (2001)* reported that CLA had no effect on feed conversion and weight gain in pigs fed with the addition of CLA. The discrepancies between the different studies on the effects of dietary CLA may be due to the different fattening stages in which the substitution of the CLA was done, also because of the composition of the different CLA isomers used in food, nutrition duration, sex of the animals, weight of pigs at the beginning of the experiment, genotype and etc.

Table 1. Summary of published reports on the effect of CLA on fattening performance of pigs

Reference	CLA (%)	Control oil/fat	Weight range (kg)	Breed[1]	Sex	ADG[2]	GF	FI
Dugan et al. (1997)	2	Sunflower oil	62-106	L x LW	♀ + ♂	nc	↑	↓
Ostrowska et al. (1999)	0,7-5,5	Soybean oil	57-107	LW x L	♀	nc	↑	nc
Thiel-Cooper et al. (2001)	0,07-0,6	No added fat	26-114	(YxL)x(DxH)	♂	↑	-	nc
Ramsay et al. (2001)	0,17-1,34	No added fat	20-55	Y x L	♀ + ♂	nc	nc	-
Wiegand et al. (2002)	0,75	Soybean oil	28-115	(YxL)x(DxH)	♂	nc	↑	nc
Sun et al. (2004)	2-4	Soybean oil	64-99	D x L x LW	♂	↑	↑	-
Szymczyk et al. (2005)	0,1-0,6	Sunflower oil	61-108	L x LW	♀ + ♂	↑	↑	-
Lauridsen et al. (2005)	0,3	Sunflower oil + Animal fat	40-130	L x Y x D	♂	↑	↑	-
Martin et al. (2007)	0,6-1,2	Palm oil	70-107	LW	♀	nc	nc	nc
Barnes et al. (2012)	0,6	Soybean oil	53-103	PIC 380	♂	nc	↑	nc

nc – No change; ↑ – Increase; ↓ – Decrease.
[1] LW– Large White; Y – Yorkshire; L – Landrace; D – Duroc; H – Hampshire.
[2] ADG – Average daily gain; GF – Gain/feed; FI – Feed intake.

The influence of CLA on pig carcass composition

One of the effects of CLA, in focus of most of studies, is the effect on reduction of the amount of fatty tissue in animals. In the first experiments on rodents, it was found that CLA affects the reduction of body fat in several places in the carcass of mice, of both sexes (*Chin et al., 1994; Park et al., 1995*). Reduction in the quantity of fat was determined in rats (*Azain et al., 2000*), hamsters (*De Decker et al., 1999*) and chickens (*Szymczyk et al., 2001*) fed with CLA. The effect of CLA on share of fat tissue in the body of animals depends on the amount of added CLA, age of animals, duration of feeding and/or genotype, which could explain these differences between studies *(Park et al., 2005)*.

In the first experiment that was done on pigs, *Dugan et al. (1997)* have found that a diet with CLA increases the share of muscle tissue and reduces the content of subcutaneous fat tissue. In this regard, *Ostrowska et al. (1999)* report the decrease in the fat : muscle tissue ratio in pigs in the final stage of fattening fed CLA at various doses (up to 5.5 g CLA/kg feed). The above authors have found that the degree of decrease of the amount of fat tissue in the pig carcass to be positively correlated with the proportion of CLA in food used in a fattening.

One of the main indicators of carcass quality of slaughter animals is the thickness of subcutaneous adipose tissue (*Stanišić et al., 2012c*), a number of researchers have found that the pig nutrition with CLA leads to its reduction (*Dugan et al., 1997; Eggert et al. , 1999; Ostrowska et al., 1999; Thiel-Cooper et al., 2001)*. However, some researches state that CLA did not affect the change in thickness of fat (*Dunshee et al., 2002 Gatlin et al., 2002; Lauridsen et al., 2005*). In addition to the effect of CLA on fat metabolism and reduction of fat tissue content depend on its concentration in the feed used in the fattening, it also depends on the "leanness" of pigs used in the experiment. In this regard, *Dunshee, et al. (2005)* suggest that the diet with CLA has a greater effect on pigs of fatty breeds than meat genotypes.

Table 2. Summary of published reports on the effect of CLA on carcass composition of pigs

Reference	CLA (%)	Control oil/fat	Weight range (kg)	Breed[1]	Sex	FT[2]	F	M
Dugan et al. (1997)	2	Sunflower oil	62-106	L x LW	♀ + ♂	↓	↓	↑
Ostrowska et al. (1999)	0,7-5,5	Soybean oil	57-107	LW x L	♀	-	↓	↑
Thiel-Cooper et al. (2001)	0,07-0,6	No added fat	26-114	(YxL)x(DxH)	♂	↓	-	-
Ramsay et al. (2001)	0,17-1,34	No added fat	20-55	Y x L	♀ + ♂	↑	-	-
Wiegand et al. (2002)	0,75	Soybean oil	28-115	(YxL)x(DxH)	♂	↓	-	-
Sun et al. (2004)	2-4	Soybean oil	64-99	D x L x LW	♂	↓	-	-
Szymczyk et al. (2005)	0,1-0,6	Sunflower oil	61-108	L x LW	♀ + ♂	↓	↓	↑
Lauridsen et al. (2005)	0,3	Sunflower oil + Animal fat	40-130	L x Y x D	♂	nc	nc	nc
Martin et al. (2007)	0,6-1,2	Palm oil	70-107	LW	♀	nc	-	-
Barnes et al. (2012)	0,6	Soybean oil	53-103	PIC 380	♂	↓	-	-

nc – No change; ↑ – Increase; ↓ – Decrease.
[1] LW– Large White; Y – Yorkshire; L – Landrace; D – Duroc; H – Hampshire.
[2] FT – Fat tickness; F – Fat tissue; M – Muscle tissue.

Conclusion

CLA is a mixture of geometric isomers of linoleic acid, which are determined by various positive effects in several animal species. Also, research has shown that CLA has beneficial effects on human health, which is why most of the current research related to CLA is aimed at regulating the feed in order to increase the level of CLA in milk, meat and other food products.

Feeding pigs with the addition of CLA is gaining increasing importance in recent years, because it affects the improvement of feed conversion, reduces thickness of subcutaneous fat and increases lean meat. However, above mentioned positive effects were not confirmed in every trial where pigs were fed diets containing CLA, because there are many factors that affect its efficiency.

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Konjugovana linolna kiselina u ishrani svinja: Efekat na rezultate tova i sastav trupa

N. Stanišić, D. Živković, M. Petrović, N. Delić, G. Marinkov, Lj. Stojanović

Rezime

Poslednjih godina izveden je veliki broj istraživanja vezanih za efekte ishrane životinja sa konjugovanom linolnom kiselinom (CLA). CLA predstavlja smešu geometrijskih izomera linolne kiseline, za koju su utvrđeni razni pozitivni efekti kao što su: uticaj na poboljšanje konverzije hrane i uticaj na smanjenje količine masnog tkiva i povećanja udela mišićnog tkiva u trupu životinja. Razlog velikog interesovanja za ovo jedinjenje su i njeni potencijalni zdravstveni efekti na ljude, kao što su antikancerogeno dejstvo, poboljšanje rada imunog sistema, smanjenje nastanka kardiovaskularnih bolesti i sl.

Ishrana svinja sa dodatkom CLA dobija sve veći značaj poslednjih godina, jer utiče na poboljšanje konverzije hrane, smanjenje debljine potkožnog masnog tkiva i povećanja mesnatosti. Međutim, svi ovi pozitivni efekti nisu potvrđeni u svakom ogledu ishrane svinja sa CLA, jer postoje više faktora koji utiču na njenu efikasnost.

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THE UTILIZATION OF PROBIOTIC BACTERIAL STRAINS FOR MONOGASTRIC ANIMALS WITHIN THE EUROPEAN UNION

G. Biagi, C. Pinna

Department of Veterinary Medical Sciences, University of Bologna, 40064, Ozzano Emilia, Italy
Corresponding author: giacomo.biagi@unibo.it
Invited paper

Abstract: The microbiota of the hindgut plays a very important role in maintaining the state of health of the gastrointestinal tract and, generally speaking, of the host. The utilization of specific dietary supplements containing viable probiotic bacteria might positively influence composition and metabolism of the intestinal microbial population. In farm animals, the use of probiotics has been reported to improve the immune system efficiency, increase resistance against enteropathogenic bacteria, and improve feed utilization and growth performance. When administered to companion animals such as dogs and cats, probiotics may stimulate the immune system and improve gastrointestinal health. In Europe, probiotics are classified as zootechnical feed additives and can be marketed only after specific authorization. Today, building a registration dossier to prove the safety and efficacy of a probiotic strain is very expensive and time-consuming. As a consequence, only a few strains (mainly Gram-positive bacteria belonging to the types *Bacillus*, *Enterococcus*, *Lactobacillus* or *Pediococcus*, and certain yeast strains) are present on the European market as feed additives to be used in animal nutrition. This paper presents an overview of the scientific literature dealing with the use of probiotics both in farm and companion animals.

Key words: probiotics, intestinal microbiota, monogastric animals, European legislation

Introduction

The gastrointestinal microbiota is a complex ecosystem made up of hundreds of bacterial species, some of which are potentially pathogenic, while others are considered good for the host *(Roberfroid et al., 1995; Gaggìa et al., 2010)*. The beneficial microorganisms that reside in the large intestine influence gastrointestinal functionality and the host's health in general, in virtue of some principal mechanisms: 1) detoxification of some toxic substances introduced

through the diet or newly formed as a result of metabolic processes of the body and of intestinal microbiota *(Tomomatsu, 1994)*; 2) "barrier effect" against the proliferation of potentially pathogenic bacteria and their adhesion to the intestinal mucosa, thanks to occupation of the attack sites of these microorganisms and production of selective antimicrobial substances *(Liévin-Le Moal and Servin, 2006)*; 3) uptake of ammonia and amines used as a source of nitrogen to support microbial protein synthesis, with a consequent reduction in the intestinal absorption of these undesirable substances *(Howard et al., 2000)*; 4) interaction with the host immune system *(Round and Mazmanian, 2009; Cerf-Bensussan and Gaboriau-Routhiau, 2010)*; 5) production of vitamins *(LeBlanc et al., 2012)*.

It has been shown that an increase of the beneficial bacteria that reside in the gut is a way to treat various intestinal disorders and maintain host health *(O'Hara and Shanahan, 2007)*. A probiotic has been defined as a „live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance" *(Fuller, 1989)*. More recently, probiotics have been defined as „live microorganisms, which when administered in adequate amounts, confer a health benefit on the host" *(FAO/WHO, 2002)*. The simultaneous combination of probiotic strains and a source of prebiotic molecules that they can metabolise might offer the administered bacterial strains greater possibilities of growing and colonising the host, thus promoting the potential beneficial effects. The combination of a prebiotic substance and one or more probiotic bacterial strains is defined as a synbiotic *(Schrezenmeir and De Vrese, 2001)*.

Modes of action and effects of probiotics

Efficacy of probiotics is influenced by many factors, including their metabolic activities and ability to survive along the gastrointestinal tract; moreover, type of probiotic used, dose, timing and length of administration are all factors that can affect efficacy of probiotic treatment *(Gaggìa et al., 2010)*. It has been shown that administration of probiotic bacteria can result in increased resistance against enteropathogenic bacteria. In fact, probiotics can reduce the risk of intestinal colonization by pathogens by producing short-chain fatty acids *(Servin, 2004)*, releasing antimicrobial peptides (bacteriocins; *Mazmanian et al., 2008*), producing enzymes that hydrolyze bacterial toxins *(Gillor et al., 2008)* and competing with pathogens for the same nutrients and sites of adhesion *(La Ragione and Woodward, 2003)*. Moreover, probiotics interact with the host defenses and may enhance immune response *(Salzman et al., 2003)*.

In pigs, the utilization of probiotics has been studied mainly in the post-weaning phase. In fact, weaning is a very stressful period that can have a dramatic impact on piglet immune status leading to microbiota imbalance and high

incidence of gut disorders and diarrhea *(Modesto et al., 2009)*. In weaned pigs, improved growth performances and intestinal health conditions have been reported in animals receiving probiotic strains of *Enterococcus faecium (Zeyner and Boldt, 2006)*, *Lactobacillus rhamnosus* GG *(Zhang et al., 2010)*, *L. plantarus (Konstantinov et al., 2008)*, *Bifidobacterium animalis (Modesto et al., 2009)*, *Bacillus* spp. strains *(Alexopoulos et al., 2004; Bhandari et al., 2008)* and live yeasts *(Bontempo et al., 2006)*. Similarly, benefits from the consumption of probiotics were observed in broilers administered with strains of lactobacilli *(Hofacre et al., 2003; Kalavathy et al., 2003)*, bifidobacteria *(Jung et al., 2008)*, *Bacillus cereus* / the relatively high number of studies that showed benefits from the administration of probiotics to monogastric farm animals, effects of probiotics on growth performances of pigs *(Chu et al., 2011; Kenny et al., 2011)* and poultry *(Estrada et al., 2001; O'Dea et al., 2006)* are contradictory. A possible reason for these discrepancies might be that utilization of probiotics in industrial pig and poultry production is problematic as loss of probiotic viability might occur as a consequence of environmental factors and feed processing techniques.The utilization of probiotics has gained attention also in the nutrition of companion animals. When administered to dogs, there is evidence that *Lactobacillus* spp. probiotic strains might exert positive effects on animal intestinal health, reducing the intestinal number of some potentially harmful bacteria and concentrations of toxic metabolites *(Baillon et al., 2004; Biagi et al., 2007)*. In a study with cats, *Biagi et al. (2013)* observed that dietary supplementation with a combination of a *Bifidobacterium pseudocatenulatum* strain and galacto-oligosaccharides (1% of the diet) resulted in higher faecal counts of bifidobacteria and lower faecal concentrations of ammonia and other protein catabolites of bacterial origin. In a study by *Benyacoub et al. (2003)*, administration of a strain of *Enterococcus faecium* to young dogs enhanced animal immune function. In humans and laboratory animals, probiotics have shown anticancer properties *(Donaldson, 2004)* and some evidence exists that probiotics (as well as prebiotics and synbiotics) might be helpful in the prevention and treatment of food allergy *(Del Giudice et al., 2010)*. Nevertheless, at present, there is still a lack of scientific literature about these subjects in companion animals.

Regulation and safety assessment in the EU on probiotics in feed

According to the Regulation (EC) No 1831/2003, in Europe, probiotics are classified as zootechnical feed additives and can be marketed only after specific authorization. In order to be marketed within the EU, a probiotic strain must be on a positive list which clearly identifies the bacterial strain and target animals (species and age) and provides information about minimum and maximum levels

of inclusion in the complete diet and period of authorization. For a probiotic strain to be authorized, the EU guidelines require that a registration dossier is compiled by the manufacturer; unfortunately, this process can be very expensive and time-consuming.

The registration dossier consists of three main sections:

1) Identity and quality of the probiotic strain, including strain main characteristics (taxonomy, metabolism, …);
2) Safety: probiotic strains must be harmless for the target animal species, the handler, the consumer and the environment; safety includes lack of pathogenicity, genotoxicity and mutagenicity, oral toxicity, toxin production and antibiotic resistance;
3) Efficacy: probiotic efficacy must be demonstrated for the target species (through at least three significant studies in two different locations). Information must be provided regarding the target species, the conditions (age, physiological stage, type of production), the usage doses, the claimed performances as well as the possible action mechanisms.

The registration dossier is sent to the European Commission that passes it to EFSA (***European Food Safety Authority***) which relies on a specialized experts panel (FEEDAP) for its scientific assessment. EFSA has introduced the concept of Qualified Presumption of Safety (QPS; *EFSA, 2005*), presumption being defined as "an assumption based on reasonable evidence" and qualified to allow certain restrictions to apply. When a probiotic strain belongs to a taxonomic unit already granted with the QPS status, the only requirements in relation to the safety assessment comprise identity of the strain, evidence that strains are not excluded by any of the qualifications imposed for the particular taxonomic unit and product-specific safety data. At present, three taxonomic groups of microorganisms have been considered to be suitable for the QPS status *(EFSA, 2007)*: gram-positive non-sporulating bacteria (GPNS), *Bacillus* spp. and yeasts. The GPNS group comprises bifidobacteria and several lactic acid bacteria but *Enterococcus* spp. was excluded because *Enterococcus* strains are amongst the leading causes of community- and hospital-acquired (nosocomial) infections. Similarly, most *Bacillus* spp. strains received the QPS status but *B. cereus* was excluded as it has been long recognized as an agent of ***food poisoning***. Today, due to the high costs that are involved in building a registration dossier to market a new probiotic strain, only a few strains (mainly Gram-positive bacteria belonging to the types *Bacillus*, *Enterococcus*, *Lactobacillus* or *Pediococcus*, and certain yeast strains) are sold in Europe as animal feed additives. The European Union regularly updates the Register for Feed Additives that contains the positive list of probiotic strains to be used in animal feeding. Despite the progress in the legislation for the evaluation of probiotics that has been made in Europe, USA and Canada, some recent studies clearly showed

that commercial probiotic products might not contain the organisms cited on the labels or even contain other bacterial species *(Mattarelli et al. 2002; Weese and Arroyo, 2003; Wannaprasat et al., 2009)*. Moreover, the number of viable bacteria found in the probiotic products might be considerably lower than declared *(Wannaprasat et al., 2009)*.

Conclusion

In conclusion, evidence exists that probiotics might have a positive effect on growth performances and health of pigs and broilers and these effects seem to be more evident in young and stressed animals. Nevertheless, probiotic efficacy can be negatively affected by many factors, including environmental conditions and feed processing techniques. When administered to dogs and cats, probiotics may result in improved intestinal health and immune status. There is some evidence for the possible role of probiotics in the prevention of food allergy and some types of cancer in humans and experimental animals but there is a lack of scientific literature about these subjects in companion animals. Today, building a registration dossier to prove the safety and efficacy of a probiotic strain is very expensive and time-consuming. As a consequence, only a few strains (mainly Gram-positive bacteria belonging to the types *Bacillus*, *Enterococcus*, *Lactobacillus* or *Pediococcus*, and certain yeast strains) are present on the European market as animal feed additives.

Korišćenje probiotskih bakterija u ishrani monogastričnih životinja u okviru Evropske Unije

G. Biagi, C. Pinna

Rezime

Gastrointestinalna mikrobiota je kompleksan ekosistem sačinjen od stotina vrsta bakterija, od kojih su neke potencijalno patogene, dok se druge smatraju dobrim za domaćina. Korisni mikroorganizmi koji se nalaze u debelom crevu imaju uticaj na gastrointestinalnu funkcionalnost i zdravlje domaćina uopšte, na osnovu nekih osnovnih mehanizama: 1) detoksikacija nekih otrova, 2) "barijera efekat" protiv širenja potencijalno patogenih bakterija 3) prihvatanje amonijaka i amina koji se koriste kao izvor azota za podršijalne sinteze proteina, 4) interakcija sa imunološkim sistemom domaćina, 5) proizvodnja vitamina. Probiotik je definisan

kao "živi mikrobakterijski dodatak hrani koja blagotvorno utiče na životinju domaćina tako što bi unapredila crevni balans".Korišćenje posebnih dodataka ishrani koji sadrže održivih probiotske bakterije može pozitivno uticati na sastav i metabolizam crevne mikrobakterijske popilacije. Kod svinja i živine, upotreba probiotika (uglavnom enterokoke, laktobacila, bifido bakterije, bacili i živi kvasac) poboljšva efikasnost imunološkog sistema, povećava se otpornost protiv bakterija enteropatogenih, i poboljša iskorišćenost hrane i performanse rasta. Ipak, uprkos relativno velikom broju studija koje pokazuju koristi od primene probiotika u ishrani preživara, efekti probiotika na performanse rasta svinja i živine su kontradiktorni. Kada se primenjuje na male životinje kao što su psi i mačke, probiotici mogu da stimulišu imuni sistem i poboljšaju gastrointestinalno zdravlje. Postoje neki dokazi za moguću ulogu probiotika u prevenciji alergije na hranu i neke vrste raka kod ljudi i eksperimentalnih životinja, ali postoji nedostatak naučne literature o ovim temama kod kućnih ljubimaca.

U Evropi, probiotici su klasifikovani kao zootehnički aditivi i mogu se stavljati u promet samo posle posebne autorizacije. Danas, izgradnja registracionih dosijea da bi se dokazala bezbednost i efikasnost soja probiotika je veoma skup i dugotrajan postupak. Kao posledica toga, samo nekoliko sojeva (uglavnom gram-pozitivnih bakterija koje pripadaju vrstama *Bacillus, Enterococcus, Lactobacillus* ili *Pediococcus*, i određenih sojeva kvasca) su prisutni na evropskom tržištu kao aditivi za hranu za životinje.Registracija dosijea se sastoji od tri glavna dela, u vezi identiteta, bezbednosti i efikasnosti soja probiotika. Registraciona dokumentacija se dostavlja Evropskoj komisiji i prosleđuje se EFSA (***European Food Safety Authority***) koja se oslanja na specijalizovane panele stručnjaka (FEEDAP) za naučne procene koja se zasniva na konceptu kvalifikovane pretpostavke bezbednosti (Qualified Presumption of Safety - KPS). U ovom trenutku, tri taksonomske grupe mikroorganizama se smatraju pogodnim za KPS status: Gram-pozitivne ne sporulišuće bakterije (uglavnom bakterije mlečne kiseline i bifido bakterije), *Bacillus* spp. i kvasci.

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THE ROLE OF LIGHT IN BROILER PRODUCTION

Z. Škrbić[1], Z. Pavlovski[1], M. Lukić[1], V. Petričević[1], D. Milić[2], G. Marinkov[1], Lj. Stojanović[1]

[1]Institute for Animal Husbandry, 11080, Belgrade-Zemun, Republic of Serbia
[2]Perutnina-Topiko, 24300 BačkaTopola, Republic of Serbia
Corresponding author: zdskrbic@gmail.com
Invited paper

Abstract: Light is an important and complex environmental factor which is often used as a management technique in order to improve the welfare of broilers and production efficiency. The complexity of light is based on three aspects of action: the intensity, wavelength, i.e. colour, length and distribution during the 24-hour cycle. The effect of light is important to establish the secretory patterns of melatonin and the formation of the circadial rhythm of essential metabolic processes and the normal functioning of the immune system. Traditional application of continuous lighting is in the function of manifestation of the genetic potential of broilers for rapid growth, while lighting programs that include periods of darkness are used as a means of improving welfare. The objective of the present study is to present the results of previous researches in this area, given the large number of variations of lighting programs, various aspects of the influence of light, relevancy from the standpoint of the welfare of broilers. Performed researches indicate that broilers reared in conditions of sufficient dark rest periods have fewer health problems associated with metabolic disorders and sudden infant death syndrome, diseases and deformities of the skeleton. On the other hand, restricting lighting programs reduced body weight, have adverse effects on the conformation and carcass yield. Defining of the age of broilers when the dark periods can be introduced, the optimum length of uninterrupted dark period, and determination of the type of alternative lighting program (reduced, intermittent, "step-up") should be based on a detailed consideration of interaction effects of lighting program with genotype and sex of chickens, and other environmental factors, significant for productivity and welfare.

Key words: lighting, broiler performances, carcass quality, welfare

Introduction

Light is an important environmental factor and management technique, based on the exposure of broilers to different modes, which have the potential to change the behaviour and physiological pathways and thus can have significant

effects on production and welfare of broilers. The effect of light consists of three different aspects: intensity, duration and wavelength. The intensity of light and colour play a role in behavior modification, and the length of the photoperiod is important for the health of broilers (*Olanrewaju et al., 2006*). Lighting programs in broiler production are numerous and vary according to the length and distribution of photoperiod within 24-hour cycle.

Traditionally used continuous (24L) or near-continuous (23L: 1D) light programs provide maximum time for food consumption aimed at rapid and intense growth of broiler chickens. Studies have shown that the use of continuous light deprived broiler rest and caused physiological stress (*Campo and Davila, 2002*). Elevated levels of plasma corticosterone and increased ratio of heterophils and lymphocytes are the most widely accepted physiological indicators of stress. Too long photoperiods may adversely affect the functional development of the eyes, the occurrence of cannibalism (*Lewis and Gous, 2009*). Light is crucial for the occurrence of diseases that are associated with a rapid increase of broilers. So, in these lighting programs the frequency of occurrence of sudden infant death syndrome and skeletal diseases is increased (*Classen et al., 1991*). The introduction of lighting programs with longer dark periods is beneficial to the welfare of broilers, based on clear patterns of day and night that indicate separate periods of rest and periods of intense activity during the day. Generally, by shortening of the photoperiod early growth of chickens is reduced, also their susceptibility to metabolic and skeletal diseases is reduced which contributes to improvement of their health, of the food efficiency but reduces carcass yield and changes the carcass conformation, which contributes to lower breast yields, higher leg yields and unpredictable changes in the content of abdominal fat.

Given the large number of variations of lighting programs, various aspects of light perception and relevancy considering the welfare of broilers, the aim of this paper is to present an overview of the results of previous researches in this area and the role and importance of light in the management of broiler production.

The physiological basis for effects of light

The primary biological rhythms of poultry, daily and seasonally, are mediated by light. The significance of light to stimulate the secretion of hormones that largely control the growth, maturation and reproduction of birds is known. Biological rhythms are present at all levels of the organization, sub cellular, cellular, physiological, as well as at the level of individual behavior. Melatonin, the pineal gland hormone, is involved in the formation of circadian rhythm of body temperature, essential metabolic processes that affect the pattern of intake of food and water and the process of digestion, secretion of lymphokines which are integrated into the normal function of the immune system (*Apeldoorn et al., 1999*). Daily periods of darkness are necessary for the establishment of normal secretory

patterns of melatonin, given the pineal gland photosensitivity. Melatonin is released during the dark period in response to the activity of serotonin-N-acetyltransferase, an enzyme that catalyzes the synthesis of melatonin in the pineal gland, while the light inactivates the enzyme. The concentration of plasma melatonin during the periods of darkness was significantly higher than in the period of light, as opposed to chickens exposed to constant light, where there is no established diurnal rhythm of melatonin secretion (*Ozkan et al., 2006*). Melatonin, in addition to the specific role in establishing the rhythm and synchronization of physiological and chemical processes in the body, hormone levels, and metabolic processes during feeding and digestion, has a protective role - protecting cells from the action of free radicals. Chickens that are grown under conditions with sufficient dark periods have fewer health problems associated with metabolic disorders and sudden infant death syndrome, diseases and deformities of the skeleton (*Apeldoorn et al., 1999; Sanotra et al., 2002 Petek et al., 2005)*, considering that the mineralization of bones is one of the processes affected by diurnal rhythms. Biological rhythms are usually coordinated with external cycles and tend to oscillate in constant conditions. Chickens are able to maintain constant normal diurnal rhythm, capable to organize patterns of behavior, such as diet, rest and movement in relation to the day and night (*Sanotra et al., 2002*).

Production performances and carcass quality

Light program as an integral part of the production technology, has to be adjusted, particularly, to the characteristics of the facility, broiler age, nutrition and targeted final body weights. Depending on the type of the facility, i.e. whether it is fully closed or open with access/windows for natural light, it is possible to achieve varying degrees of light control program. Application of continuous or near-continuous lighting program is a function of the maximum manifestation of the genetic potential of broilers for rapid growth. However, results of recent studies (*Schwean Lardner et al., 2012*) show that broilers decreased physical activity with extension of the photoperiod, linearly at young age and squared at a later age, and the most time spent on food is in the moderate photoperiod of 17L. Comparing lighting programs of varying durations of photoperiod (12L:12D, 16L:8D and 20L: 4D) confirmed that the longer periods of darkness prevented regular access to food, which leads to less consumption and limited the growth of broilers (*Classen et al., 1991)*. The main objective of restrictive lighting program is slowing down the initial growth to reach physiological maturity before the maximum increase in muscle mass. It is necessary to provide a sufficiently long production cycle for the expression of compensatory growth of broilers which eliminates the negative effect of reduced photoperiod on body weight. Given that today's broiler production tends to shorten the fattening period, compensatory growth is often missing. Lighting programs that provide manifestation of compensatory growth of broilers are based

on gradual extending of photoperiod, so called "step-up" lighting programs. The positive effects of their use can be influenced or dependant on genotype or interaction effects of genotype x lighting program (*Škrbić et al., 2012*).

Intermittent lighting programs (IL), which include alternating shifts of light and dark periods, increase the body weight of broilers (*Škrbić et al.,* 2007), regardless of the total length of the photoperiod, which may be shorter than the discontinuous restrictive lighting programs (*Ingram et al., 2000*). In intermittent lighting programs broilers consume food during the light period, until they are full, and due to reduced physical activity do not consume too much energy during the dark periods, resulting in greater gain. According to *Gordon (1999)* there is a change in the rhythm of feeding, so that the peak of the food consumption is reached at the beginning and end of the light period. Greater efficiency of food intake was confirmed in restrictive (*Meluzzi et al., 2003*) and intermittent lighting programs (*Petek et al., 2005; Rahimi et al., 2005*) in relation to continuous light program. Short, limited periods of light used for consumption contribute to better feed conversion rate and longer dark periods for food digestion. Improvement of the health status of broilers in conditions of long periods of darkness reflected on the mortality rate, which is significantly higher in conditions of continuous lighting program (*Shah and Petersen, 2001*). Linear decrease of mortality with extending of the period of darkness was determined by *Schwean Lardner et al. (2013).* Restrictive lighting program (12L:12D) compared to intermittent-restrictive lighting programs (12 (1L:1D) and 2 (6L:6D)) result in lower mortality of broilers (*Classen et al., 2004)*, however, in other studies (*Ingram et al., 2000; Škrbić et al., 2012*) the effect of lighting program on mortality was not observed.

Few studies have focused on the conformation and yield, i.e. carcass quality in conditions of restrictive lighting. Keel length and shank length, generally are considered to be good indicators of development of the skeleton which is related to the development and the amount of muscle mass. In conditions of restrictive lighting (12L:12D) *Ingram et al. (2000)* have found a significantly lower shank length as compared to controls (23L:1D), which indicates a decrease in body weight of broilers, while the length of the keel was not significantly influenced by the photoperiod. Sex of the chicken is important in respond to changes in lighting program (*Škrbić et al., 2011*). Namely, the authors noted a significantly better body development of female chicks, expressed by index values of conformation measures, in conditions of intermittent programs compared to continuous lighting. Improvements ranged from 0.9 for thigh girth, up to 2.1 index units for shank length.

The positive effect of constant light on carcass yield was determined, in the research by *Lien et al. (2007)* it was on average by 0.5% higher than in the treatment with restricted lighting. In comparison of continuous and "step-up" lighting program (*Downs et al., 2006*) no significant difference in carcass yield and share of abdominal fat in broiler chickens was confirmed, while *Škrbić et al.*

(*2012*) in the examination of the "step-up" program and lighting program of moderately-constant photoperiod have noted significant interaction effect of lighting program and genotype on yield of classically dressed carcasses and ready to grill carcasses. There is a concordance in results on the occurrence of the substitution between the share of breast and legs in BW%, when the share of breast is reduced in the restricted (*Lien et al., 2007*), "step-up" (*Downs et al., 2006)* and discontinuous (*Škrbić et al., 2008*) lighting program in relation to the continuous program. The reason could be the reduction of early growth and occurrence of compensatory growth that compensated weight, but through growth of carcass parts that normally grow earlier, such as legs and wings, and lagging behind of late-growing parts of the carcass, such as the breast meat.

Broiler welfare

The primary importance of light in broiler production is in the field of broiler welfare. In addition to the duration and distribution of photoperiod, for the welfare, in terms of physical activity and exercise of fundamental behaviors, important are also the intensity and color of light.

Chickens reared under continuous light were exposed to stress as evidenced by a higher ratio of heterophils and lymphocytes, and show the fear response, according to the specified time of tone immobility compared to chickens reared under the regime of 14L:10D (*Campo et al., 2007*). Lighting programs that include periods of darkness are used as a means of improving the welfare of broiler chickens because they provide time for rest, which reduces stress. Shortening of the photoperiod reduces susceptibility to metabolic diseases (*Petek et al., 2005*), thanks to the establishment of normal secretory patterns of melatonin, while intermittent lighting can reduce problems with legs and the incidence of lameness (*Christensen et al., 2004*), bringing the total mortality and mortality due to metabolic and skeletal diseases down linearly with increasing periods of darkness (*Schwean Lardner et al., 2013)*. Reduced early growth of broilers in conditions of shortened photoperiod enables normal development of the skeleton in the absence of burden caused by large muscle mass. Also, there is a change in physical activity of broiler chickens, especially if they are exposed to intermittent lighting program, which contributes to the health and quality of bones (*Škrbić et al., 2009*). Some authors (*Petek et al., 2005*) do not associate directly the occurrence of skeletal diseases with the duration and distribution of photoperiod during the day they believe that the weight of broilers, which is limited by ration type nutrition in the intermittent lighting programs, has a decisive role, and is negatively correlated with the incidence of problems with legs.

Gait score is accepted methods of welfare assessment, assuming that lameness of broilers, though accompanied by pain in the legs, makes it difficult for broilers to access food and water and prevents the satisfaction of basic

physiological needs. Broilers spend in average 76% of the time during the day in continuous light lying, and in lame broilers with estimated gait score 3, the inactivity increases to 86%. Also, the number of visits to the feeders reduces from 50 to 24 (*Weeks et al., 2000*). In conditions of moderate photoperiod the frequency of occurrence of gait score > 2 significantly reduces compared to continuous lighting (*Sanotra et al., 2002*). Exposure of broilers to intermittent lighting programs has beneficial effect on the condition of the legs, however, significant differences in regard to gait score occur also depending on the design of the lighting program or the length and frequency of periods of darkness and light. *Classen et al. (2004)* have found significantly higher values of gait score in conditions 2 (6L: 6D) and 12 (1L: 1D) compared to 12L: 12D.

Behavior of broilers is under the direct influence of light intensity. Generally, the more intensive light stimulates activity of broilers and reduces the incidence of skeletal disease, while light of lower intensity can be used to control aggressive behaviour and prevent cannibalism. Recommendations for light of low intensity (less than 5 lux) are based on the assumption that in this way feed conversion is improved, mortality due to sudden death syndrome and injuries and damage to the carcass reduced due to less activity, have not been confirmed by results of scientific research. On the contrary, the use of extremely low intensity light (1 lux) had a negative effect on the welfare of broilers, reducing their activity, expression of the basic behaviours and increasing occurrence of ulcerative foot-pad lesions (*Deep et al., 2010*). Some authors have found the lack of significant effect of the intensity and source of light on the health of the legs, gait score, foot-pad and hock burns (*Kristensen et al., 2006*). Colour of light of a specific wavelength, has a variable stimulatory effect on the retina, resulting in the change of behaviour that may affect growth and development (*Lewis and Morris, 2000*). In this regard, the blue light has a calming effect and can be used to stimulate growth, while red light due to higher wavelengths penetrates more easily to receptors in the hypothalamus and is important for the stimulation of reproductive traits. Chickens exposed to white light were more active in walking, pecking, stretching wings, and chickens exposed to red light, showed more aggressiveness. On the basis of the free choice of light colour after the age of 28 days, it was found that chickens prefer blue or green light, regardless of the colour of light under which they were previously grown (*Prayitno et al., 1997*).

Conclusion

Overall, despite a number of previous studies and deriving results on the effects of light, due to its different aspects (duration and distribution of photoperiod, intensity, colour, etc.), the impact on opposing objectives in broiler

production, i.e. the production performance and welfare, it is difficult to compare the lighting programs and to define the optimal one.

Lighting programs that include periods of darkness for the broilers to rest certainly are means to improve broiler welfare. However, based on the confirmed decrease in body weight, adverse changes in conformation and carcass yield in these lighting programs, the question of the optimal duration of uninterrupted dark period remains open, as well as the definition of age of broilers for the introduction of the dark period, in order to avoid negative effects on the performance of broilers; opting for type of alternative lighting program (reduced, intermittent, "step-up"), should be based on a detailed consideration of interaction effects of lighting program with genotype and sex of chickens, and other environmental factors that are significant for productivity and welfare.

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Uloga svetlosti u brojlerskoj proizvodnji

Z. Škrbić, Z. Pavlovski, M. Lukić, V. Petričević, D. Milić, G. Marinkov, Lj. Stojanović

Rezime

Svetlost predstavlja važan i kompleksan faktor sredine koji se sve češće koristi i kao tehnika menadžmenta u cilju poboljšanja uslova za dobrobit brojlera i efikasnosti proizvodnje. Kompleksnost svetlosti zasnovana je na tri aspekta delovanja: intenzitet, talasna dužina, odnosno boja, dužina i distribucija u toku 24-časovnog ciklusa. Uticaj svetlosti je važan za uspostavljanje sekretorne šeme melatonina i formiranje cirkadijalnog ritma esencijalnih metaboličkih procesa i normalne funkcije imunog sistema. Tradicionalna primena kontinuiniranog osvetljenja je u funkciji ispoljenosti genetskog potencijala brojlera za brzi porast, dok se svetlosni programi koji uključuju periode mraka koriste kao sredstvo za poboljšanje dobrobiti. Cilj rada je prikaz rezultata dosadašnjih istraživanja u ovoj oblasti, obzirom na veliki broj varijacija svetlosnih programa, različite aspekte uticaja svetlosti, aktuelnost sa stanovišta dobrobiti brojlera. Sprovedena istraživanja ukazuju da brojleri gajeni u uslovima dovoljnih mračnih perioda za odmor imaju manje zdravstvenih problema u vezi sa metaboličkim poremećajima i

sindromom iznenadne smrti, oboljenjima i deformitetom skeleta. S druge strane, restriktivni svetlosni programi redukuju telesnu masu, nepovoljno utiču na konformaciju i prinos trupa. Definisanje uzrasta brojlera za uvođenje mračnih perioda, optimalne dužine neprekidnog mračnog perioda, kao i opredeljivanja za vrstu alternativnog svetlosnog programa (redukovan, intermittent, "step-up") treba da je zasnovano na detaljnijem sagledavanju interakcijskih efekata svetlosnog programa sa genotipom i polom pilića, kao i drugim faktorima sredine, značajnim za produktivnost i dobrobit.

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ALTERNATIVE GROWTH PROMOTERS AND BROILER'S INTESTINAL MORPHOLOGY AND FUNCTION

D. Žikić, G. Uščebrka, S. Stojanović, Z. Kanački

University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000, Novi Sad, Republic of Serbia
Corresponding author: dragan.zikic@stocarstvo.edu.rs
Invited paper

Abstract: The use of antibiotic growth promoters in broiler diets has been almost eliminated within EU market from a combination of EU legislation and consumer pressure. A large number of alternatives has been developed or are under development. Despite the need for further research into the impact on the broiler performances, recent research and development of growth promoters has been focused on functional benefits including resistance to gastrointestinal bacterial infection, antibacterial activity, and improved immune status in broiler chicks. Important for understanding the effects of alternative growth promoters have the studies of their impact on the morphological and functional characteristics of the digestive tract. The objective of this paper was to present effects of alternative growth promoters on broiler's intestinal structural and functional characteristics through review of research publishing in this field.

Key words: broiler, growth promoters, gut morphology

Introduction

Antibiotics have been widely used in animal production for decades. Antibiotics are used therapeutically to improve the health and well-being of animals, for prophylactic purposes and to improve productive performance (growth rate, feed conversion) as antimicrobial growth promoters or AGPs. The use of antibiotic growth promoters in broiler diets has been almost eliminated within EU market from a combination of EU legislation (EC Regulation No. 1831/2003) and consumer pressure.

AGP removal has led to animal performance problems, feed conversion increases, and a rise in the incidence of certain animal diseases, such as (subclinical) necrotic enteritis *(Dibner and Richards, 2005*). Decreased production results and the risk of disease outbreaks have compelled the researchers to explore

the utility of other non therapeutic alternatives (*Perić et al., 2009*). The focus of alternative strategies has been to prevent proliferation of pathogenic bacteria and modulation of indigenous bacteria so that the health, immune status and performance are improved. A large number of alternatives has been developed or are under development, including probiotics, prebiotics, novel carbohydrate fraction, acidifiers and phytogene additives.

Though a lot of research was done on the modes of action of AGPs, less is known about mechanisms of action for alternatives. Gastrointestinal tract can adapt and react morphologically and functional to some factors related to changing the diets as addition of AGPs or alternatives for AGPs. The intestine can change its surface by growing to length, and/or by increasing or decreasing the height of its villi. The increased villi height and decreased crypt depth indicate that addition of alternatives growth promoters can have a beneficial effect on digestibility. It is suggested that usage of growth promoters could reduce both the damage of enterocytes and the need for cell renewal in the gut. A high cell turnover occurs at the epithelium of the small intestine of chickens (*Sklan, 2001*), which is accompanied by an extremely high rate of metabolism, involving 23 to 36% of the whole body energy expenditure (*Summers, 1991*).

Because of the fact that alternative growth promoters have different mechanisms of action, aim of this paper is to present for each group individually, mode of action and describe changes of broiler's intestinal morphology and function.

Probiotics

Probiotics have been defined as 'mono- or mixed cultures of living microorganisms which beneficially affect the host by improving the properties of the indigenous microbiota'. Existing probiotics can be classified into colonizing species and free, non-colonizing species *(Žikić et al., 2006)*.

The positive effects of probiotics on the performance of broilers are reflected in broiler growth, improvement of feed conversion and reduced mortality *(Timmerrman et al, 2006; Li et al., 2008; Bjedov et al., 2009; Tabidi et al., 2013)*. On the other hand, no positive results could be established in application of probiotic preparations in fattening of broilers in studies by certain number of researchers *(Bittencourt et al., 2011; Dizaji et al., 2012*). Wishing to explain in a scientific way inconsistent results which they obtained in their studies, majority of authors concluded that the effect of probiotics depended on the combination of bacterial strains contained in the probiotic preparation, stability during feed manufacturing (as well as in the gastro-intestinal tract), level of its inclusion in the mixture, composition of mixture, conditions of the environment in the production

facility, variation in the physiological state of the bird, and the actual microbiota balance in the gut of the animal (*Huyghebaert et al., 2011*).

The mechanism of action of probiotics as AGP replacers will depend on the nature of the organism and is not always clear. The different bacterial species in the normal microbiota (colonizing on the epithelium of the digestive tract or occurring freely in the gut lumen) of the broiler gut reach a typical equilibrium state after about a week post-hatch, and depends on many factors including location in the gastro-intestinal tract, integrity of the intestinal mucosa and transit time of the chymus (*Teirlynck et al., 2009*). *Brown* (2011) suggest the following mechanism of action of probiotics: enhance of epithelial barrier integrity, competitive exclusion, interference with quorum sensing signaling agents, secretion of bacteriocins, anti-inflammatory effects and immunomodulation.

Research conducted by *Uščebrka et al.* (2005b) and *Perić et al*. (2010) point out that probiotics have significant affect at the morphology of the intestinal wall. *Uščebrka et al.* (2005b) point out that usage of probiotic culture in broiler diet significantly increase villi height in duodenum, jejunum and ileum compared with control group. Also, significant decrease of crypt depth was found in duodenum and jejunum of chickens with supplementation of probiotics in diet. Villus height / crypt depth ration was increased in probiotic groups compared with control group in all three regions of small intestine. The gut morphology examination showed that probiotics had beneficial effect on jejunum morphology causing a significant increase in villus height and villus surface area compared to control group (*Perić et al., 2010*). According to these results, *Awad et al.* (2010), point out that supplementation of the probiotic product in broilers' diet increased villus height and villus height / crypt depth ratio in duodenum and ileum. *Samanya and Yamauchi* (2002) also reported increased villus height and villus height / crypt depth ratio of duodenum in birds supplemented with the probiotic product. Intestinal morphology including duodenal and ileal villus height and crypt depth as well as villus height / crypt depth ratio are indicative of gut health in broilers. Increased villus height and villus height / crypt depth ratio are directly correlated with an increased epithelial turnover (*Fan et al., 1997*), and longer villi are correlated with activation of cell mitosis (*Samanya and Yamauchi, 2002*).

Supplementation of the multi-microbe probiotic products in broiler diets resulted in increased villus height and villus height / crypt depth ratio in duodenum and ileum at day 35 (*Kim et al, 2012*). On the other hand, supplementation of multi-microbe probiotic product in different concentration in broiler diets resulted in no significant differences among treatments regarding villus height, crypt depth and villus height/crypt depth ratio in duodenum and ileum (*Tsirtsikos et al, 2012a*). On the contrary, probiotic inclusion level resulted in increasing cecal villus height and crypt depth.

Prebiotics

A prebiotic was defined as non digestible food ingredients that beneficially affect the host, selectively stimulating the growth or activity, or both, of one or a limited number of bacteria in the colon (*Gibson and Roberfroid, 1995*). Prebiotics are a source of carbon and energy for the friendly strains of bacteria already inhabiting in the colon, where bacterial fermentation processes of some nutrients occurs. Their advantage compared to probiotics is that they promote growth of useful bacteria which are already present in the host organism and are adapted to all conditions of the environment (*Yang et al., 2009*).

Results of the effects on broiler performance are contradictory. In analysis of the effects of implementation of fructo-oligosaccharides (FOS) on broiler performances it was established that improvement of gain and improvement of feed conversion *(Li et al., 2008; Yang et al., 2009)*. But, *Biggs et al.* (2007) obtained research results showing decrease of gain by 2% in group fed FOS in diet.

Results obtained in studies carried out at the Faculty of Agriculture in Novi Sad indicate positive effect of Bio-Mos on performance of broiler chickens (*Perić et al, 2005; Žikić et al., 2008*), but it is necessary to emphasizes that obtained differences for investigated parameters weren't statistically significant (*Perić et al., 2009*).

The results of a number of authors have shown that the use of prebiotics in the diet of broilers significantly alters the structure and function of the digestive tract. The most important changes are in the intestines and small intestines are where the most significant changes in the structure. Structural changes are primarily related to mention the length of the various parts of the intestine, but also in changing the structure of intestinal wall. The most frequently monitored parameters are the villus height, crypt depth, villus height /crypt depth ratio, villus area, changes in the number of goblet cells. Our research shows that MOS significantly affect on the intestinal structure of broilers (*Uśćebrka et al., 2005a*). *Žikić et al.* (2011) point out that the addition of Bio-Mos in the broiler diets, resulted in a decrease of crypt depth in week one. Our results are in agreement with the report of *Yang et al.* (2008) who pointed out that in their trial, in the 1st week of broiler's age, villus height was not statistically different between Bio-Mos fed broilers as compared to the unsupplemented group, but differences between groups were established in the crypt depth. Significant differences in the jejunal parameters were found from the 21 days of age until the end of the trial. Our results are not in accordance with the results of *Sun et al.* (2005) who stated that age of birds plays an important role in the changes in intestinal morphology, whereas micronutrients may have an effect on intestinal structure development after 35 days

of age. In an experiment with Bio-Mos, *Iji et al.* (2001) observed that Bio-Mos significantly increased jejunal villi height. Addition of MOS to the feed of 14 days old poults had significant effects on jejunal villus height /crypt depth ratio as compared to the control group (*Ferket et al., 2002*). These changes were represented by elongated villi and a higher villus/crypt ratio, which was indicative of a lower rate of enterocyte-cell migration from the crypt to the villus. It was suggested that Bio-Mos could reduce both the damage to enterocytes and the need for cell renewal in the gut. *Houshmand et al.* (2012) point out that MOS significantly increase duodenal villi height at 21 and 42 day, while jejunum villi and also duodenum and jejunum crypt depth were not affected. *Baurhoo et al*. (2007) found that birds fed diet containing a prebiotic had longer villi than those fed the control diet. In a study conducted by *Xu et al.* (2003), dietary addition of a prebiotics (FOS) significantly increased villus height. They suggested that these changes may be related to the ability of FOS to create a more favourable intestinal microbial environment and are not a direct effect of FOS on the intestinal tissue.

Natural carbohydrate fraction (NCF) is a new yeast-based feed ingredient isolated from a specific strain of yeast. *Brümmer et al.* (2010) explored the effects of NCF on broiler chickens. They found a comparable effect to that observed with commercial doses of MOS when using 20 times lower concentrations of NCF. In a study conducted by *Ivković et al.* (2012), NCF did not have the expected effect on improving the integrity of the gut. The strongest effect noted for NCF-fed birds was the enlargement of the goblet cell cup size. An enlargement of goblet cell cup size was clearly observed in this trial, as in the previous reported work with NCF (*Brümmer et al., 2010)* and has also been observed in trials with MOS (*Baurhoo et al., 2009*). Greater cup size means that there was more mucin in the cell, which can be the result of greater mucin synthesis or slower mucin secretion.

Phytogenic feed additives

Phytogenic feed additives (PFA) are plant extracts derived from herbs or spices, which have beneficial effect on animal production and health. A large variety of the plants have properties which could potentially improve feed intake, digestion, feed conversion and body weight gain (*Ertas et al., 2005; Stainer, 2009*). On the other hand, some authors reported no effects on body weight gain and feed intake (*Lee et al., 2003; Jamroz et al., 2005; Nasir and Grashorn, 2010; Amad et al., 2011; Amad et al., 2013*) or feed conversion ratio (*Ocak et al., 2008*). These discrepancies may be due to numerous factors such as type and parts of plants used, their physical properties, time of harvest, the preparation method of PFA and their compatibility with other feed components (*Jang et al., 2007*). The active molecules include many different secondary plant metabolites, resulting in a broad

range of physiological effects, like secretolytic and spasmolytic, or immune-stimulative effects (*Lee et al., 2004*) and mucus secretion (*Tsirtsikos et al, 2012b*). The mode of action of these feed additives is not completely clear. They have antimicrobial, antiviral, antioxidant and many other biological activities (*Ertas et al., 2005; Cross et al., 2007; Al-Kassie, 2010*). They act as a digestibility enhancers, stimulating the secretion of endogenous digestive enzymes (*Lee et al., 2003*). *Lee et al.* (2003) and *Jang et al.* (2004) indicated that essential oils used as feed additives for broilers enhanced the activities of trypsin and amylase. Recently, it was suggested that dietary spices induce changes in cell membrane fluidity and permeability properties, resulting in increased absorption of micronutrients from the small intestine (*Prakash and Srinivasan, 2010*).

Perić et al. (2010) point out that commercial phytogenic blend (essential oils derived from oregano, anis and citrus, and fructooligosacharides) had no significant effect on villus height or villus surface area, but significantly reduced the villus height /crypt depth ratio in broiler jejunum. In broilers fed diets supplemented with PFA (consists of a mixture of essential oils with thymol and anethole as leading active substances) there were no differences between the jejunal villus height and crypt depth could be observed compared to the control. Villus height / crypt depth ratio was significantly increased in birds fed PFA compared to the control (*Amad et al, 2013*). Addition of PFAs were no significant differences between treatments regarding villus height, crypt depth and villus height / crypt depth ratio in the duodenum and ileum of 14-day-old broilers (*Tsirtsikos et al., 2012b*). No differences between treatments were observed in 42-day-old broilers for all intestinal segments examined. Increasing dietary PFA inclusion level showed a pattern of linear increase of duodenal villus height / crypt depth ratio in 14-day-old broilers and ileal villus height in 42-day-old broilers.

Ocak et al. (2008) reported that adding dry peppermint or thyme leaves as growth promoters in broiler feed had no effect on the relative weights of the whole gut, pancreas and addible inner organs. That indicates that the improved production results obtained in broilers fed the phytogenic additive are not directly connected with the improved gut morphology, but with other mechanisms. It was reported that active principles of herbs, essential oils or plant extracts may act as digestibility enhancers, balancing the gut microbial ecosystem and stimulating the secretion of endogenous digestive enzymes and thus improving growth performance in poultry (*Cross et al. 2007*).

Conclusion

Withdrawal of antibiotics from poultry feed created need for alternative solutions which would influence improvement of health and production traits of

broiler chickens. Numerous products are on the market, and while some products clearly have potential, for others the efficacy is not clear. All product classes described in this paper have potential to be alternatives to AGPs. Our previous studies indicate that the tested alternatives have significant influence on the structure and function of the intestine of broilers, which leads to improved production results. But, our results point out that PHA positive effects on productive performance are not due to changes in the morphological structure of broiler's intestinal wall.

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Alternativni promoteri rasta i morfologija i funkcija creva brojlera

D. Žikić, G. Uščebrka, S. Stojanović, Z. Kanački

Rezime

Upotreba antibiotskih promotera rasta u ishrani brojlera je isključena sa tržišta EU zbog zakonske regulative i pritiska potrošača. Veliki broj alternativa je razvijen i dalje se razvija. Zbog potrebe daljih istraživanja vezanih za uticaj na proizvodne performanse brojlera, trenutna ispitivanja i razvoj promotera rasta su fokusirana na funkcionalne benefite koji uključuju otpornost digestivnog trakta na bakterijske infekcije, antibakterijsku aktivnost i poboljšanje imunološkog statusa kod brojlerskih pilića. Od velike važnosti za razumevanje efekta alternativnih promotera rasta imaju istraživanja o njihovom uticaju na morfološke i funkcionalne karakteristike digestivnog trakta. Cilj ovog rada je da prikaže efekte alternativnih promotera rasta na morfološke i funkcionalne karakteristike creva brojlera kroz pregled publikovanih istraživanja u ovoj oblasti.

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BIOTECHNOLOGICAL SOLUTIONS FOR THE GROWTH STIMULATION OF BROILERS

D. Šefer [1], S. Radulović [2], R. Marković[2], D. Jakić Dimić [3], D. Milić [4]

[1] Department of Nutrition and Botany, Faculty of Veterinary Medicine, University of Belgrade.
[2] Department of Botany and Nutrition, Faculty of Veterinary Medicine, University of Belgrade.
[3] Scientific Veterinary Institute of Serbia, Belgrade.
[4] Perutnina, Ptuj, Topiko
Corresponding author: dsefer@vet.bg.ac.rs
Invited paper

Abstract: "Biotechnology" is a term that includes the complementary use of biology and technology in livestock production. In order to managed to maintain, feed industry, because of the more significant changes in habitual and edible habits of the people, as well as new regulatory requirements, should respect the opinion of the consumer and try to offer a safe animal products whose production does not pollute the environment. As a basic tool of biotechnology during the past decade has imposed the introduction of alternative solutions that are intended to improve feed efficiency and consequently improve production results of farmed animals. In order to stimulate growth in broilers, commonly have been used enzymes, chelating forms of trace elements and in recent years more attention is paid to the use of probiotics. The aim of the adding the enzyme is addition to activity of endogenous enzymes of animals, elimination of anti nutritional factors, increasing energy and nutritional value of feed and decreasing the excretion of less usable nutrients in the environment. Today of practical importance are enzymes of NSP and cellulolytic complex, protease, amylase and phytase. Our trials have shown that the use of amylase allows the use of meals with lower energy content (5%) with no negative impact on performance and also lowers the value of consumed feed per kilogram of gain. Use of phytase in the quantity of 1000FU/kg can replace about 30% of the total, or 50% of available phosphorus in diets for broilers without significant changes of production results. Probiotics are ecological way of control pathogenic bacteria and the ability to stimulate the growth by using physiological potentials and mechanisms of animals. The results of our research with the use of different bacterial cultures have shown the positive effects on weight gain with less consumption of feed and consequently better feed conversion. Beside the inorganic forms of minerals today are increasingly used so-called "chelating" forms, or organically bound trace elements. It was observed that the minerals associated with the amino acid or peptide is better protected during the passage through the stomach to the site of absorption than inorganic salts. Results

obtained in testing of using organically bound selenium pointed to the positive impact on the increase in carcass weight and meat yield of broilers that received chelating form of selenium. Summing up the obtained results of investigations that have been carried out, we are able to draw the conclusion that the use of growth promoters as a biotechnology solution in broilers diet has its nutritional, medical and economic justification.

Key words: broilers, biotechnology, growth stimulants, probiotics, enzymes

Introduction

"Biotechnology" implies the complementary application of biology and technologies in livestock production. In order to managed to maintain feed industries, due to the pronounced changes in habitual and edible habits of the people, as well as new regulatory requirements, should respect the opinion of consumers and try to provide a safe animal products whose production does not pollute the environment. Modern intensive livestock production places heavy demands in front of the organism of domestic animals where one of the imperatives of industrial animal keeping is to achieve as lower feed consumption per unit gain in the shortest possible time. The production of broiler meat during the last years records a rise in the world and represents 85.56% of the total poultry meat production (*Bigili*, 2002). Based on the evaluation, production of chicken meat should be noted a further increase due to several factors-good conversion compared to other species, it is accepted by all cultures and religions, a small amount of fat and a large quantity of meat, from a health point of view attractive properties, low price, fast and quick reproductive cycle. According to estimates of Anonymous & FAO (2002) it is expected in 2015 to produce 100 million tons and in 2030 even 143 million tons of chicken meat. However, it is unrealistic and impossible to expect optimal performance in production if it is not successful and adequate solved the issue of their nutrition. Diet must contain all the necessary nutrients that will satisfy not only the needs of animals to sustain life, but also for their production (*Šefer*, 2002). As a basic tool of biotechnology during the past decade has imposed the introduction of alternative solutions that are intended to improve feed efficiency and consequently improve production results of farmed animals. To get a better feed efficiency, longer preservation, easier handling, and ultimately increase production and improve the quality of food of animal origin, beside the basic nutrients, feed mixture contains a number of additives that have different purposes. Additives are substances added into the meal in small quantities,

emphasizing useful and suppressing harmful effects (*Sinovec* i Šefer, 2004). Recently, special attentions of the scientific community, and certainly the consumer, have caused growth stimulants. Data of the results of using different growth promoters are incomplete and often contradictory, which increases the importance and timeliness of this issue. In broilers nutrition as growth stimulants commonly are used enzymes and chelate forms of microelements and during the recent years, increased attention is paid to the use of probiotics.

Application of enzymes in broilers nutrition

Enzymes (former ferments) are protein catalysts of chemical reactions in the organism and other biological systems. By the texture they are proteins and have a role to accelerate the chemical reactions, needed in small quantities and they do not deplete during the reaction. Enzymes help the complex chemical reactions in the body to conduct strictly controlled at relatively low temperatures (37 ° C or lower), with nearly neutral pH. The importance and effectiveness of using the enzymes in food stems are not only from the knowledge of physiological characteristics of nutrition of individual species and categories of animals, but also the knowledge of forms and amounts of certain nutrients and anti-nutritional substances in the plant feed. Enzymes, as well as the positive effects of their use in order to improve the nutritional value of meals have been known for years. Today, as food additives, of practical importance are enzymes of cellulolytic e complex (cellulase and pectinase), NSP complex (β-glucanase, xylanase, β-galactosidase), protease, amylase and phytase.

In human and animal nutrition the most important polysaccharide is starch. It is a major reserve nutrient of plant organisms and also the main source of energy in the diet of broilers. Grain contains about 60-80% starch and legumes 40% (Šefer and Sinovec, 2008). Starch in plants is in the form of small granules (cells) in which there are hydrogen bonds which make them resistant to water penetration and hydrolytic enzymes that break it down by hydrolysis. *Noy and Sklan (1995)* found that the ileal digestibility of starch is about 85%. Several important parameters affects the digestibility of starch (*Tester and Karalas 2006):* structure of the starch, form of granule, content of amylose, the amount of double helices in the starch grain, size and a mixture of granules. *Helbert et al. (1996)* have identified several steps in the breakdown of starch by α amylase:

1) Diffuse deployment of the enzyme on the surface of granules.
2) Start of hydrolysis.
3) The centripetal hydrolysis leading to the formation of the channel pores that leading to the central part of the grain.

4) Centrifugal degradation of granules which starts by enzymes breaking through inside it.

Exact data on effects of using α-amylase are few, and to a large extent contradictory.

Zanella et al. (1999) claimed that the digestibility of starch can be increased by the use of enzymes in 37-day-old broilers by 1.8%. *Noy and Sklan* (*1995)* reported that the secretion of amylase in the duodenum of broilers is low in the first 4 days of life and gradually increases up to 21 days. On the other hand *Uni et al. (1995)* found that the secretion of amylase in the duodenum of broilers is also low in the first 4 days of life, but it only grows up to 7 days after which the secretion of amylase is stabilized. *SL Vieira et al. (1994)* carried out the experiment on Cobb provenance of broilers where the control group was fed a diet of standard raw and chemical composition. Experimental groups were fed identical diets as the control group, but the content of energy was less than the control by 60, 90 and 120kcal/kg. Experimental group which had the energy value less for 120kcal/kg were added the 200, 300 and 400 g / t Ronozyme A (200 kilo α-amylase units / gram). Adding of enzymes fully compensated lower energy value of the meal. *Gracia et al. (2003)* performed an experiment in order to examine the impact of adding amylase on production performances of broilers fed diets based on corn and soybeans. Adding enzyme α-amylase in broiler ration in the experimental group resulted in greater weight gain (4.7%) improved consumption (3,78%) and a lower conversion (1,3%) of food compared to the control group of broilers. The aim of the experiment conducted by *Pavlović (2008)*, which was set up under the guidance of Department of Nutrition and Botany, Faculty of veterinary medicine (FVM) in Belgrade, was to monitor production performances and health of broiler chickens fed diets with standard raw and chemical composition and the diets with reduction of energy followed by the addition of α-amylase. The experiment was conducted on a total of 100 broilers, divided into two equal groups of 50 animals. The first group of chickens (control) was fed with complete feed mixtures for fattening broilers based on standard raw components and chemical composition, without enzymes. The second group (experimental group I) was fed diets in which the energy content was decreased by 5% with the addition of the enzyme α amylase 40 KNU per kg of complete feed mixture. In the middle of the experiment (21. day) broilers of the experimental group I have achieved significantly lower ($p < 0,01$), body weight (698,40 g) compared to broilers of the control group (745,40 g). At the end of the experiment (42 days) broilers fed diets added with α-amylase (experimental group I) achieved numerically higher body weight (2241g) compared to the control group of broilers (2193g) without statistically significant differences ($p > 0,05$). During the first phase of the experiment (from 1 to 21 days) experimental group I achieved a lower weight gain (33,26 g) compared to the control group (35,45 g), but the difference was statistically significant ($p < 0,001$) . In the second phase (from 21 to 42 days) that experimental group fed a diet

supplemented with α-amylase (experimental group I) achieved higher daily weight gain (73,24 g) compared to the control group of broilers (69,01 g), with differences that were highly significant (p <0,001). Differences in body weights and daily gains showed that the largest effect of addition of α-amylase activity were expressed during the second period of fattening (21.- 42. day) that coincides with the results of *SL Vieira et al. (1994), Gracia et al. (2003)* and *Yuste et al. (1991)* who found that the digestive tract in 21 day old chicks is not fully developed and able to digest starch so that there is a good reason to add α-amylase in the later stages of broilers life. Throughout the entire experiment from 1 to 42 days (*Pavlovic*, *2008)* experimental group of broilers fed diets supplemented with α-amylase (experimental group I) achieved higher average daily gain (53,25 g) compared to the control group of broilers (52,23 g) without statistically significant differences (p> 0,05). Observed for the entire experiment, broilers fed diets in which the energy content was decreased by 5% with the addition of amylase achieved better conversion (1,827) compared to the control (1,837). These results are consistent with the findings of *SL Vieira et al. (1994), Gracia et al. (2003)* who found that the addition of α-amylase in broiler diet improved feed conversion.

The use of selenium and vitamin E in the diet of broilers

Selenium is an essential element in the poultry nutrition and participated in protection against exudative diathesis and pancreatic fibrosis, and supports the activity of glutathione peroxidase. Selenium together with Vitamin E represents a multi-component system that protects biological membranes from oxidative degeneration. The recommended concentration of vitamin E in diets for broilers is 15-20 mg / kg feed. Numerous studies have shown that the use of significantly higher amounts of vitamin E is an effective way to improve the quality and sustainability of broilers meat (*Janssens,* 1998). Daily needs for selenium during the intensive farming is 0,15 mg / kg (*National Research Council NRC*, *1994*). Selenium which is used as an additive in vitamin mineral premixes can be in one of two basic forms: organically bound to amino acids or as inorganic salt (usually sodium selenite). The richest sources of selenium are inorganic sources, or certain salts of selenium (selenite, selenate). Beside the inorganic forms, often is used organically bound selenium ("chelated"). Organically bound selenium has two important advantages:
Animals can deposit it for periods when the need for it increases (eg, when selenium deficient diet or stress). Selenium content of meat increases along with the addition of Se-amino acids, which includes it in the food chain of people.

Adding selenium in substrate resulted in production of the so-called "selenium yeast" in which the most of Se is incorporated into the Se-methionine, selenium form that is easily absorbed by all animal species (Marković et al., 2011). Great number of researchers have confirmed in their experiments reasonable

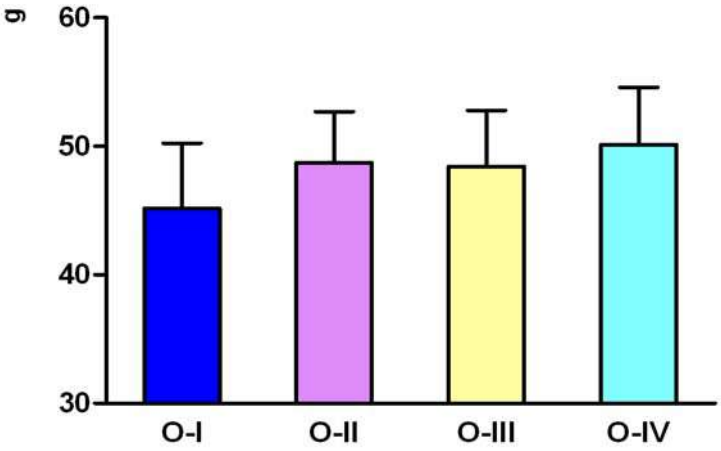
substitute of inorganic form with organic selenium (Sel -Plex), showed by better results in the production of broilers. *Edens* (*2001*) in trials with broiler chickens showed that the final body weight at 42. day in the control group (food containing selenium in an amount from 0,28 mg / kg without subsequent addition of selenium) was 2,38 kg. After the addition of inorganic selenium (0,2 mg / kg) body weight was 2,43 kg, and after the addition of Sel-Plex (0,2 mg / kg) it was 2,45 kg. The positive effects of Sel-Plex on conversion confirmed the trials of *Edens et al*., (*2001*). Effect of organic selenium (0,15 ppm) on the growth performances and mortality have been showen by a *Stolić et al*. 2002) in experiments on broilers. Growth rate was better by 4,2% and conversion by 9,8% when used Sel-Plex, compared to the group that received the same amount of sodium selenite in feed. The experiment in Brazil (*Arruda et al., 2004*) performed at 2400 Ross broiler hybrids (1-42 days old), adding 0.1 ppm selenium (Sel-Plex) in combination with 0,2 ppm selenium (as sodium selenite) resulted in an improved growth and conversion in comparison with the group of broilers that received 0,3 ppm of sodium selenite in feed. Highest body weight, weight gain and the best conversion was obtained in experiments in which broilers were supplemented with 12,50 mg / kg Se and 300 IU of vitamin E (*Swain et al., 2000*). The combination of 0,3 ppm organic selenium and 250 mg / kg of vitamin E affected the mortality reduction, the positive effect in stressful conditions and improved production performance in broilers (*Roch et al*., *2000*). In the experiment (M*arkovic*, *2008)* which was organized by the Department of Nutrition and Botany FVM in Belgrade, the main task of the study was to determine the effect of feeding broilers diets with different sources of selenium and different amounts of vitamin E on the performance and health of broilers. The trial lasted 42 days and included a 240-day old chickens of Cobb 500 provenance, both sexes, average initial body weight of 41,25 ± 2,97g divided into four equal groups of 60 animals in each. All groups were fed with standard raw and chemical composition feed mixtures predicted for a given provenance with the difference in the amount and type of additive, as shown in Table 1.

Table 1. Content of selenium and vitamin E in the diets, [mg / kg DM feed]

Groups	Selenium		Vitamin E
	Sodium selenite	Sel-Plex	
Experimental -I	0,3	-	20
Experimental -II	-	0,3	20
Experimental -III	0,3	-	100
Experimental -IV	-	0,3	100

At the end of trial (42. day) the average body weight of broilers of the experimental group-I was 1938,46 ± 215,16 g and was significantly lower ($p < 0,01$) compared to

the average weight of broilers in the experimental groups II, III and IV (experimental group-II 2087,74 ± 168,68 g, experimental group-III 2079,09 ± 184,39 g and experimental group-IV 2146,79 ± 189,48 g). There were no significant differences (P> 0,05) between the average weight of broilers of II, III and IV experimental group. Daily weight gain of broilers is shown in Figure 1. During the all stages of fattening, experimental group II, III and IV achieved a statistically significant (p <0,05) to highly significant (p <0,01), higher weight gain compared to the experimental group-I. These results are in agreement with the statements of many authors (*Anciuti et al., 2004 Srimongkol et al.*, *2004; Edens and Gowdy, 2004*).



* O-I, O-II, O-III, O-IV (experimental group-I, experimental group-II, experimental group-III, experimental group-IV, respectively)

Figure 1. Daily weight gain of broilers during the experiment 1-42nd day [g]

Feed conversion of broilers in experimental groups II, III and IV was lower than in the experimental group-I during all phases of the experiment. The experimental group-IV achieved the best feed conversion (2,01 kg of feed per kg of weight gain). Broilers of experimental groups II, III and experimental group-I had a higher conversion (1,49%, 3,48%, 5,97%, respectively) compared to the broilers of experimental group-IV. The results of the conversion are in agreement with the results *Anciuti et al. (2004), Naylor et al, (2000), Edens and Gowdy (2004)*.

The use of probiotics as growth promoters.

Probiotics were originally described as micro-organisms and substances which contribute to the maintenance of the intestinal microbial balance and "eubiosis" *(Lilly and Stillwell, 1965)*. Subsequent definitions considered probiotic as supplements of live microorganisms that cause beneficial effects in animal hosts by maintaining eubiosis, which excluded antibiotics from this term *(Parker, 1974; Fuller, 1977; Vanbelle et al., 1990)*. Lately, more often is used the term DFM (direct fed microbial), which implies a source of viable microorganisms, including bacteria, fungi and yeasts *(Milles and Botwalla, 1991)*. In gastrointestinal tract develops a large number of bacteria, which may be characterized as a protective, supportive, neutral or harmful *(Hakkinen et al., 1997)* Probiotics represents environmentally friendly way to control pathogenic bacteria and possibility of stimulating the growth based upon physiological potential and mechanisms of animal. By using the probiotics it can be achieved similar effects as with the use of antibiotics without possible side effects such as residues, waiting period, resistance, allergies, genotoxicity, etc.(Šefer et al., 2011). Beneficial microflora acts in two ways in the digestive tract: antagonistic activity against pathogenic bacteria and competitive exclusion. Antagonistic activity of lactic acid bacteria toward pathogens is possible thanks to the production of bactericidal substances such as bacteriocins, organic acids and hydrogen peroxide *(Gilland and Speck, 1977)*. Competitive exclusion (CE - competitive exclusion) is a complex of interplay of microbes, nutrients and host factors that selectively prevent specific groups or genera / species / strains of microorganisms that inhabit the intestinal tract *(Stavric et al., 1985; Blenkenship et al., 1990)*. Beside the competitive mechanisms, *Dunham et al (1993*) consider that the poultry feed diet supplemented with the *L. reuteri* has longer villi and deeper crypts in the ileum, which affect the intensification of activity of T-lymphocytes and increases the synthesis of IgM antibodies against Salmonella. *Nahashon et al. (1994a, b)* adds that the use of lactobacilli increases cellularity of Payers plate in ileum through which stimulates the immune system of the mucosa, which corresponds to antigen stimulation by production of immunoglobulins (IgA). Some experiments conducted on broilers heve suggested that probiotic products increased the growth, improved feed conversion and significantly reduced mortality *(Tortuerro, 1973, Tortuero et al., 1989, Watkins et al., 1982, Han et al., 1984; Meluzzi et al., 1986; Owings et al., 1990; Mohan et al., 1996; Marković et al.,2008)*. By examining the effect of adding culture *L. Acidophilus* to the feed for broilers on production results it has been established an increase in daily gain. With the identical consumption broilers achieved slightly better feed conversion *(Petersen, 1998)*. It should be noted that some studies with probiotics passed without positive results in broilers *(Watkins*

and Kratzer, 1984; Maiolini et al., 1992) and laying hens *(Goodling et al., 1987)*. In a series of experiments *(Jin et al., 1996a; 1997)* were identified the positive effects of the use of monocultures or a mixture of lactobacilli and other bacteria on the growth performances of broilers. The results of trials conducted on Arbor Acres broilers hybrids *(Jin et al., 1996a)* indicate that by the use of lactobacilli as a nutritional supplement broilers achieved significantly greater weight gain, with the recommended use in the amount of 0,1% DM of food. *Jin et al. (1998)* investigated the impact of adding 0,05% to 0,015% adhesive *Lactobacillus* cultures in the mixture for broilers on growth performances. They found a significant increase in body weight of broilers (1,91 vs. 1,98-2,08 kg) and decreased feed conversion ratio (2,00 vs. 1,74-1,88 kg) by adding probiotics in the amount of the 0,05% and 0,10% but not by using a feed mixture with 0.15% (1,93 and 1,95 kg), which is in accordance with results of other authors *(Kim et al., 1988; Jin et al., 1996a; Mohan et al., 1996; Yeo et al. 1997)*. *Newman (1999)* reported the results of experiments performed in order to investigate the effect of probiotics on the performance of broilers. Using *L. acidophilus* in the amount of 0,1% DM of feed broilers have accomplished a higher body mass by 1,1% and improved feed conversion by 0,6%. Statistical differences in mortality between the groups were not determined, but the mortality was numerically lower in the treatment group. *Radakovic* (2001) set the trial under the guidance of the department of nutrition and botany, FVM in Belgrade with the aim of determining the effect of different type of probiotics on the growth performances of broilers. For the experiment it was used 204 one-day old broiler chickens Arbor Acres provenance, both sexes, average body weight 40,07 ± 0,33 g, divided into four equal groups of 51 individuals in each. The first group of chickens (control) was fed with complete feed mixtures for fattening broilers, made of standard raw components and chemical composition without the addition of probiotics, while the experimental group, in order, received the same meal as the control but with the addition of certain probiotics: Probios, Paciflor and Bioplus - 2B as presented in Table 2.

Table 2. Contents of different probiotics in diets, %

Probiotic, %	group			
	Control group-K	Experimental group -I	Experimental group -II	Experimental group - III
Probios	-	0,10-0,05	-	-
Paciflor	-	-	0,01-0,005	-
Bioplus – 2B	-	-		0,05

At the end of the experiment all experimental groups achieved higher body weight of 1,00 to 4,36% compared to the control group-K. When looking at the daily weight gain for the entire trial, 1-42. day, we can see the better weight gain in the experimental groups compared to the control and these differences were not statistically significant (p> 0,05) with the best results in Experimental group -III (50,70 g) compared to Control group-K (48,50 g). The results are consistent with the results of Jin et al. (1998) who investigated the impact of adding 0,05% to 0,015% adhesive cultures of *Lactobacillus* in the mixture for broilers on the growth performances and find out a significant increase in body weight of broilers (1,98-2,08 vs. kg 1,91) and decreased feed conversion ratio (1,74-1,88 vs. 2,00). With regards to the daily feed consumption for the entire experiment 1.- 42. day, Experimental group-I (99,52g), Experimental group-II (105,00g) and Experimental group-III (103,81g) had a lower consumption compared to the control group-K (105,48g). The movement of food conversion in the experiment during the all stages is presented in Table 3.

Table 3. Trend of feed conversion per phases of the experiment (kg)

Period of the experiment (days)	groups			
	Control group-K	Experimental group-I	Experimental group-II	Experimental group-III
1-21	1,75	1,60	1,69	1,80
21-35	2,19	2,20	2,20	2,07
35-42	2,79	2,29	2,62	2,34
1-42	2,17	2,03	2,13	2,05

In the last stage of fattening, as well as during the entire experiment all experimental groups acomplished better feed conversion ratio compared to the control group of broilers, (1.90 up to 6.50%), which is consistent with the findings of most researchers dealing with a given problem *(Tortuerro , 1973, Tortuero et al., 1989, Watkins et al., 1982, Han et al., 1984; Meluzzi et al., 1986; Owings et al., 1990; Mohan et al., 1996; Kalavathy et al 2003).*

Conclusions

Based on the results of our own experiments set up in the Department of Nutrition and Botany FVM in Belgrade and confirmed with the results of numerous national and international authors, we can derive the following conclusions:

1. Adding α amylase enzyme allows the use of a meal with lower energy content (5%) with no negative impact on the performance of broilers.

2. Feeding broilers with complete mixture enriched with organic selenium (selenized yeast and vitamin E) leads to a significant increase in production results in all phases of it.
3. The use of probiotics in broilers nutrition has given a positive impact on performance without negative impact on health.
4. The use of enzymes, organic selenium and probiotics as a biotechnological solutions for better feed efficiency in fattening broilers have their nutritional, medical and economic justification.

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Biotehnološka rešenja u stimulaciji rasta brojlera u tovu

D. Šefer, S. Radulović, R. Marković, D. Jakić Dimić, D. Milić

Rezime

„Biotehnologija“ je izraz koji podrazumeva komplementarnu primenu biologije i tehnologije u stočarskoj proizvodnji. Da bi industrija hrane za životinje uspela da se održi, zbog sve izraženijih promena habitualnih i jestivih navika ljudi, kao i novih zakonskih regulativa, treba da uvaži mišljenje potrošača i da pokuša da ponudi bezbedne proizvode animalnog porekla čijom se proizvodnjom ne zagađuje životna sredina. Kao osnovno oruđe biotehnologije u poslednjoj deceniji se nametnulo uvođenje alternativnih rešenja koja imaju za cilj poboljšanje iskorišćavanja hrane i posledično poboljšanje proizvodnih rezultata gajenih životinja.

U ishrani brojlera u silju stimulacije rasta najčešće se koriste enzimi, helatni oblici mikroelemenata a poslednjih godina sve veća pažnja se poklanja upotrebi probiotika.

Cilj dodavanja enzima je dopuna aktivnosti endogenih enzima životinja, otklanjanje antinutritivnih materija, povećanje energetske i hranljive vrednosti hraniva kao i smanjvanje izlučivanja slabije iskoristivih hranljivih materija u spoljašnju sredinu. Danas su od praktičnog značaja enzimi celulolitičkog i NSP kompleksa, proteaze, amilaza i fitaza. Naša istraživanja su pokazala da upotreba amilaze omogućava korišćenje obroka sa nižim sadržajem energije (5%) bez negativnog uticaja na proizvodne rezultate a ujedno snižava vrednost utrošene hrane po kilogramu prirasta. Upotrebom fitaze u količini od 1000FU/kg moguće je

zameniti oko 30% ukupnog, odnosno 50% iskoristivog fosfora u smešama za brojlere bez značajnijih promena proizvodnih rezultata.

Probiotici predstavljaju ekološki način kontrole patogenih bakterija i mogućnost stimulacije rasta korišćenjem fizioloških potencijala i mehanizama životinja. Rezultati naših istraživanja pri upotrebi različitih kultura bakterija ukazuju na pozitivne efekte na visinu dnevnog prirasta uz manju konzumaciju hrane I posledično bolju konverziju hrane.

Pored neorganskih formi mineralnih materija danas se sve više koriste tzv. „helatne“ forme, odnosno organski vezani mikroelementi. Zapaženo je da su minerali vezani sa aminokiselinom ili peptidom bolje zaštićeni za vreme pasaže kroz želudac do mesta resorpcije nego neorganske soli. Rezultati koje smo dobili u ispitivanjima upotrebe organski vcezanog selena ukazala su na pozitivan uticaj na povećanje mase trupa i prinosa mesa kod brojlera koji su dobijali helatni oblik selena u hrani.

Sumirajući dobijene rezultate sprovedenih istraživanja u prilici smo da izvedemo zaključak da korišćenje stimulatora rasta kao biotehnoloških rešenja u ishrani brojlera ima svoje nutritivno, medicinsko i ekonomsko opravdanje.

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IMPORTANCE OF COCCIDIOSIS IN POULTRY PRODUCTION

S. Lilić[1], S. Dimitrijević[2], T. Ilić[2]

[1]Institute of meat hygiene and technology, Kaćanskog 13, 11000 Belgrade, Republic of Serbia
[2]Faculty of veterinary medicine, Belgrade University, Bulevar oslobođenja 18, 11000 Belgrade, Republic of Serbia
Corresponding author: slobo@inmesbgd.com
Invited paper

Abstract: Coccidiosis is a world-wide and permanent health problem in poultry production, especially in the intensive systems with large number of animals. It is the most important parasitic poultry disease as far as economy is concerned since yearly costs of prophylaxis, as well as of therapy exceed 2 billion Euros, at the global level per year. In Serbia the disease has the highest prevalence in chicken, less in turkeys, gees, ducks and pheasants. The incidence of the disease depends on the lack of space on the farm, high temperature and high relative humidity, improper feeding, other diseases and all factors that can compromise bird immunity and general resistance to infectious diseases. The cause of the infection is protozoa belonging to the *Eimeridae* family, with oocyst spores as infective form. The source of the infection are infected birds, whereas the disease can spread in the sussceptible bird population by direct and indirect contact such as dust, objects on the farm, people, rodents, wild birds, as well as insects. Coccidiosis is the disease of the spring and fall, i.e. humid seasons with plenty of rain. The parasite develops in the epithelial cells of the intestine of all bird species. The parasite can develop also in epithelial cells of the kidney glomerully in gees whereas merozoits and shizonts (as a developing form of the parasite) cause severe lesions and desquamation of the mucus. Local symptoms are accompanied with general health disturbance and typical diarrhea which is the characteristic symptom. Diagnosis is based on the clinical symptoms, pathomorphologicaly and pathohystologicaly, as well by microscopicaly in feces samples. To control coccidiosis in poultry, there is a prophylactic measures – common measures as mechanical cleaning, washing and disinfection; as well as using the vaccines and by adding the anticoccidials into the feed mixtures (coccidiostatics and coccidiocides). Economical consequences of the coccidiosis in poultry are: smaller weight gain, inadequate feed conversion, smaller body weight at the end of the fattening period, prolonged fattening period, as well as therapy costs. Body weight gain is reduced, as well as accumulation of abdominal fat. The disease has a

negative impact on chemical and sensory meat appearance. One of the problems as far as coccidiosis is concerned is drug resistance. Today, coccidiosis control strategies are the „shuttle" and „switch" program of the prophylactic medication, good manufacturing praxis and proper sanitation.

Key words: coccidiosis, poultry, importance

Introduction

Coccidiosis is the most important infectious poultry disease and represents a constant health problem, especially in intensive poultry industry. It is world-widespread and costs on yearly basis, for prophylaxis, as well as therapy exceed two billion Euros (*Dallouil and Lillehoj, 2006*). Several domestic species are susceptible, however concerning the incidence, as well as economic consequences, coccidiosis is most important in poultry, rabbits, ruminants, carnivores and less in swine. In Serbia, coccidiosis is most important in the poultry production.

Cause of the disease belong to phylum *Apicomplexa*, class *Sporozoa*, subclass *Coccidia*, ordo *Eucoccidia*, suborder *Eimerinae*, and family *Eimeridae* that has two ordo: *Eimeria* and *Tyzzeria*. Depending on the localization, disease in poultry has two forms: coccidiosis of the caecum that is caused by *Eimeria tenella* and intestinal coccidiosis that is caused by a number of parasites: *E. necatrix, E. acervulina, E. maxima, E. brunetti, E. mitis, E. mivati, E. praecox* and *E. hagani*. Coccidiosis of turkeys is caused by: *E. adenoides, E. meleagrimitis, E. gallopavonis, E. dispersa, E. inocua, E. meleagridis* and *E. subrotunda*. In gees the disease can be in the form of renal infection *E. truncata* and intestinal coccidiosis: *E. anseris*, *E. nocens*, *E. parvula* and *E. stigmosa*. Duck coccidiosis is caused by *Tyzzeria perniciosa*; however, *E. anatis* and *E. danailovi* can also cause the disease. In pheasants, coccidiosis is caused by *E. dispersa, E. phasiani, E. langeroni, E. pacifica, E. megalostomata, E. gennaeuscus, E. duodenalis, E. colchici, E. picta* and *E. tetartooimia*. In our region, *E. tenella* is highly pathogenic and the most prevalent.

In the last few years the poultry industry and as a consequence chicken meat represents 80 percent of the whole production of meat originating from birds. Still, production is the fastest growing in the meat industry. According to analysis, production, as well as consumption of chicken meat, will rise because of: good feed conversion in comparison to other animal species, there is not religious aspect of poultry meat consumption, poultry meat is healthy (low fat and high protein content), has good sensory quilities, low price and fast production which mean a short generative time. Poultry, during coccidiosis and after therapy, have poor

productive results. Daily feed quantity and feed conversion rise. Chicken daily growth weight is reduced, as well as body mass at the end of the fattening period (*Vermeulen et al., 2001*). As a result the fattening period should be prolonged. At the same time, care should be taken for the withdrowal period for the drug wich further rises costs of production (*Williams, 2002*).

Because of coccidiosis, carcass yield is smaller, as well as the proportion of more valuable parts of the body. Also, fat deposits are smaller in the abdominal fat tissue. In broilers' meat, there is higher water content and less proteins. Relative proportion of proteins of the fibrinous tissue in the total protein mass is higher. Sensory characteristics of the broilers' meat are bad in comparison to the population where coccidiosis was absent (*Lilić, 2007*). In liver of infected borilers, content of iron and copper is smaller. Meat of infected broilers have a decreased iron manganese and phosporous content (*Koinarski et al., 1998*).

A great economic problem is resistency to anticoccidial drugs. Such drugs are not easy to use. Also, development of new drug generations, that are for prophilaxis and therapy, is expensive. As an alternative, there are investigations whose target is to use immunological, biotechnical and genetical methods for prevention and control of coccidiosis (*Grag et al, 1999*). Of all coccidias that cause the disease, *Eimeria tenella* is widely distributed and serves as a gold standard in order to sequence the genetical material of the causative agent. At the same time, *E. tenella* is the first candidate for eradication (*Augustine et al., 2001*).

Poultry meat consumption, at a global level is constantly rising. So, there is a need to intensify broiler production. In such a production system, the possibility for coccidiosis is higher inspite of using anticoccidials in feed. At contrary, world trends in food production are to produce organic meat, with no drugs added to the feed. This means that the risk of coccidiosis is higher. Nevertheless, strategies to control coccidiosis are still based on prophylactic medication through feed and vaccination (*Vermeulen et al, 2001*), not to exclude good production praxis and good hygiene and sanitation.

Epidemiology

In flock, disease is spreading by direct, as well as indirect contact (*Williams, 2002*). Oocysts that are infectious could be distributed by equipment, dust, people, rodents, wild birds as well as insects (*Dimitrijević and Ilić, 2003*). *Coleoptera* spp, which are usually present in the broiler population, can serve as mechanical vectors (*Calnek, 1997*).

Prevalence and distribution is influenced by several factors: high animal density cramped ona small space, high air temperature, high relative humidity, different (especially different age) categories of birds at same place, feed change,

quality of feed, as well as all other factors that compromise resistance to the disease and general health status of the birds (*Calnek, 1997*). The highest incidence of coccidiosis is during spring and fall, especially when weather is cold and humid. The incidence is significantly smaller during hot and dry weather conditions (*Maungyai et al, 1990; Calnek, 1997; Razmi and Kalideri, 2000*). The intensity of the infection depends on the number of oocists that are ingested and the immune status of the bird (*Hofstad, 1984*). Onset of the disease depends on the age of the bird at the time of the first infection and number of passages of the infect (for one passage to be completed it is required 10 days), as well as on ability of the bird to develop proper specific immune response (*Hofstad, 1984; Ilić et al., 2003*).

Coccidiosis can be appeared in the clinical and subclinical form. The subclinical form of the disease is most frequent in six weeks old chicken and infection occurs in nearly all flocks (*Jordan and Pattison, 1996*). *Voeten* (*1987*) showed that sub clinical coccidiosis is most prominent from four to six week old chicken in the case if anticoccidials are not added to the feed. According to some authors (*Braunis, 1980; Razmi and Kalideri, 2000*), subclinical forms of the disease depend on the size of the flock. Prevalence of the subacute form of the disease is significantly higher in flocks with more than 40,000 birds in comparison to flocks with less of 10,000 birds.

Pathogenesis

Infection is by oral by feed and/or water ingesting oocists in the form of spores.. After ingestion, infectious oocysts excist, liberating the infective form: the sporozoits. Sporozoits infect epithelial cells of the intestine and kidney epithelial cells. Transfer of the sporozoits up to the locus of the primary lesion is with the help of intraepithelial lymphocytes (*Lawn and Rose, 1982; Daszak, 1999*). The pathogenic process starts during shizogonic phase of the parasite development. This process during the first generation of shizonts is negligible. However, the most pathological stadium is during the second generation of shizonts. Their development, deep in the cells of Lüberkinii glands, results in inflammation, mucus desquamation, capillary rupture and haemorrhagiae. This stadium of the disease is accompanied with severe clinical symptoms. In this stadium, possible outcome could be death of the bird. Death is a consequence of haemorrhagiae (bird can lose 60 to 80 percent of the blood volume), toxemia or as a consequence of gangrene or rupture of the intestinal wall.

When coccidiosis appear, there can be other infections such as reovirus infection, Marek disease, New Castle virus infection and infectious bronchitis virus infection. In such a case, symptoms are mixed depending on causative agents (*Ruff, 1991*). Especially in Nordic countries, there are mixed infections with *Eimeria* spp,

Cl. perfringens or *E. coli*. This is because the use of antibiotics is banned (*Van Der Stroomand Van der Sluis, 1999*).

Endogenous development of renal coccidiosis in gees takes place in tubules of the kidney. As a result, there is desquamation of the epithelia, obstruction and dilatation of the tubuli by mature gamonts. Kidneys are enlarged, there are urate salts deposits in the urinary tract, as well as kidney failure.

Parasite cycle

Developmental cycle of the parasite has two phases: endogenous and exogenous. The endogenous phase is in the animal (bird) and there are two sub-phases: shizogonia (nonsexual sub phase) and gametogonia. Shizogonia is characterized by producing one after another generations of shizonts that carry merozoits as the infectious form of the parasite (*Soulsby and Rose, 1972*). During the sexual sub-phase (gametogonia), oocysts are released and infection is spread. Exogenic phase take place out of the bird. During this phase, oocysts sporulate (sporogonia).

After ingestion (one to two hours), oocysts excyst (oocysts rupture) and sporocysts release (*Lawn and Rose, 1982*). From oocysts, by further degradation, release of the sporozoits occurs. Sporozoits attack the surface of the caecal epithelium (*Patillo, 1959; Davies et al., 1963*), penetrate the basal membrane and enter the *lamina propria mucosae* whether free or inside the macrophages. Finally, they attack epithelial cells that cover the bottom of the Lüberkinii cripts (*Lillehoj and Trout, 1993*).

From the second generation of merozoits, in most cases, microgametocytes and macrogametocytes develop. Sexual phase of the parasite development, takes place in the cells of the mucus and submucus. That phase starts from 6^{th} day of the infection (*Pellerdi, 1974*). Microgametocytes (12.4 x 8.7 μm) (*Tyzzer, 1929*), enlarge and undergo through a number of divisions resulting in microgamete development (*Davies et al, 1963*)**.** Microgamets are mobile, fusiform in the shape approximately 5 μm long with three active flagella evenly distributed on one end of the cell (*Joyner and Kendall, 1963*). Macrogametocytes are transformed into the macrogametes that have granular cytoplasm and centrally placed nucleus (*Pellerdy, 1974*). When micro- and macrogametes join they form zygote. After the fertilization phase, the macrogametes mucoproteinaceous granule that is placed on the periphery of the cell, form the outer membrane of the zygote. From that form, nonporous oocyst develops.

Once the cyst wall is formed completely the oocysts are released through feces. Prepatent period is the time from the start of the infection up to the moment when first oocysts could be found in feces and it is unique for the species. In the

case of *E. tenella,* it is up to 6 to 7 days (*Pellerdy, 1974*). The maximal number of oocysts in feces is at 10th day after infection. After that time, number of the oocysts in feces sharply decline (*Hammond and Long, 1973*).

Diagnosis

Caecal coccidiosis is diagnosed by clinical signs (not reliable), coprology, and pathomorphological and pathohistological analysis.

One of the basic symptoms that could lead to diagnosis is bloody diarrhea, as well as changes in feces appearance (*Dimitrijević, 1999*). As the disease progresses, because of the blood in feces, feces are red or resemble the color of chocolate (*Jordan, 1990*). The feathers around the cloacae are covered with bloody deposits. Feces are stained with blood. Birds that survive first few days of the infection, can survive the next 10 to 15 days. During that time, birds are thirsty and rapidly loose weight (*Calnek, 1997*). Symptoms of the disease start to appear at the time when the second generation of shizonts starts rapidly to replicate, grow, mature and release the second generation of merozoits. Second generation of merozoits causes inflammation of the subepithelial mucus, desquamation of the epithelia and capillary rupture in the caecum wall. As a consequence, bloody diarrhea occurs (*Jordan, 1990*). Thirst, anorexia, somnolentia, goose pimples, dropped wings, closed eyes, leg paralysis, pale cres and mucous membranes, enterorhagia, skin depigmentation are sings as well (*Pellerdy, 1974, Ruff, 1991*). Death usually occurs on the 5th and 6th days after infection (*Hammond and Long, 1973*). The precise cause of death is not jet clear (*Calnek, 1997*). Although bleeding and gangrene or rupture of caecum are the most important (*Hofstad, 1984*). In gees with renal coccidiosis somnolence, leg weakness, birds are reluctant to move, eyes are closed, inapetencia, thirst, whitish diarrhea, dropped wings, nervous signs, neck twisting, weight loose, and death are present.

Coprology

Coprology is performed on native samples by flotation, using concentrated solutions of NaCl. The most reliable method is to find oocysts and count them by using the McMaster method. However, it is not enough to confirm the causative agent or cause of death since death can occur before onset of oocysts in the feces (*Dimitrijević, 1999; Dimitrijević and Ilić, 2003*). Positive results only show that there is infection that is at least seven days old (*Hofstad, 1984*). In the case of renal coccidiosis of gees, oocysts can be found in feces however this finding is not enough for diagnosis, since there are difficulties to differentiate them and oocysts of the intestinal coccidias.

Patomorphological lesions

In cases of intestinal coccidiosis, the first and second day after infection, on the microscopic level (patohistology) there are focal lesions of the intestinal epithelium and small necrotic foci in the subepitelial connective tissue. Those changes are the result of first generation shizont maturation. On the third day, caecums are enlarged in diameter and there are regions with petechiae in the mucosa. The most prominent macroscopic lesions are from the fourth and fifth day after infection. It is obvious since in that period the second generation of the shizonts completely matures and on the fifth day after infection there is transformation into the second generation merozoits. Entrance of the second generation of merozoits into the healthy epithelial cells, mark the moment when haemorrhagiae of the caecum start. Such findings accompanied with heterofil infiltration of the *lamina propriae* and submucosis, as well (*Calnek, 1997*).

The intestine is shortened and the intestinal wall is thickened. The lumen is enlarged two to three times. The color is dark blue with sub serous petechiae. Mucosa is thickened; surface of the epithelium, as well as the epithelium of the Lüberkini crypts is desquamated with haemorrhagic patches. The intestinal content is watery, bright red in colour with desquamed cells, erythrocytes and plenty of coccidia in different stages of development. Later on, the content becomes thick and thecolour is changed to dark red. Gradually, fibrinous tissue encirculates the content of the intestinum, resembling gray-yellow hard cork (*Nešić, 1999*).

Sixth and seventh day of infection the content of the intestinum hardens and becames dry. Epithel regeneration is fast and can be accomplished in 10 days after infection. However, as a consequence of intensive local lesions, it is possible that the epithel never returns to the previous condition (*Calnek, 1997*). Recovery starts with the appearance of fibroblasts and angioblasts (*Pellerdy, 1952*).

Examining the intestinal wall, it can be found the plenty of parasites in different stages of maturation and development. Native sample-slide is especially useful since it shows oocysts and macrogamets (*Jordan, 1990*). The pathognomonic finding is the presence of shizonts in the material (*Calnek, 1997*).

Diagnosis is made on the basis of gross lesions in the intestinum, as well as microscopically by using the content of the intestinum as a sample (*Calnek, 1997*). Intensity of the infection can also be estimated especially if there is a doubt whether coccidias are the only cause of the fatal outcome of the disease. Intensity of infection is in proportion with the number of oocysts that were ingested and is in positive corelation to other parameters such as loss of body weight and changes in feces appearance (*Hofstad, 1984*).

Postmortem examination of gees that succumbed to renal coccidiosis are cahectic and gross lesions and are localized only in kidney. Kidneys are enlarged, circular in shape, smooth and bright at the surface, grey-white or grey-yellow in

color. Sometimes the color changes to gray-red and red-brown. The surface of the kidneys have plenty of softened foci that are white or yellow, circular and 0.5 to 1 mm in diameter. These foci are not clearly separated from rest of the kidney tissue. It is possible to find whitish stripes and petechiae (*Dimitrijević and Ilić, 2003*).

Histology

Using standard pathohistology staining procedures (hematoxillyn-eosin) different stages of parasite development can be seen (*Hofstad, 1984*). In order to differentiate and identify them, it is better to use Shiff's reagent. Polysaccharides accompained with refractary granula, as well as aggregates that form the macrogamete wall, stain bright-red (*Calnek, 1997; Nešić, 1999*). Appart of the abovementioned standard technique, there are other more specialized diagnostic methods that use monoclonal antibodies conjugated with fluorescent markers (*Calnek, 1997*).

The second shizont generation migrate deep into the lamina propria; around them, there is a strong inflammatory cell reaction with eosinophils, plasma cells and in some cases giant cells (*Hofstad, 1984*). Oocysts can be found in tissue sections, and the finding depends on the stage of the infection when the sample was taken. Oocysts can be seen in giant cells next to the muscular lamina of the intestinal wall (*Pellerdy, 1974*). The first shizont generation, that matures two to three days after infection, can be seen microscopically scattered as a wide belt. Small focal hemorhagiae and necrosis can be seen in the vicinity of blood wessels in the stratum circulare internum of the intestinal wall muscular lamina (*Jordan, 1990*).

Kidney tubuli from infected gees are dilated and filled with epithelial cells and oocysts. Ureters are dilated and filled with mucous yellow-brown mass. At some places, epithel of the renal tubuli totally dissapeared and as a consequence, there are cists filled with parasites in different stages of development and cell detritus. Around most of the tubuli, there is fibrinous tissue proliferation with a number of inflamatory cells (*Dimitrijević and Ilić, 2003; Dimitrijević and Ilić, 2011*).

Prophilaxis and therapy of coccidiosis

Disease can be treated with anticoccidials. They can act either as coccidiostatics, that inhibit growth and development of the intracellular parasite form or coccidiocides. Coccidiocides destroy the parasites during their developmental stages. Most of the anticoccidials are coccidiocides or they are at the beggining of the action coccidiostatics and in later stage, coccidiocides (*Long and Jeffers, 1986*). In order to prevent coccidiosis, it is possible to add some of the

above mentioned substances in the feed for birds. In case therapy is needed, the drug is given diluted in drinking water.

Anticoccidials are devided in 12 groups: benzenacethonitril derivatives (clazuril and diclazuril), benzyl-purin (arprinocid) derivatives, xarbanilid derivatives (nicarbazine), gvanidine derivatives (robenidin), dinitrobenzamide derivatives (dinitolmid), ionofors-polyether antibiotics (monensin, lasalocid, narasin, salinomicin, maduramicin, alboriksin), piridins (klopidol), quinazolines (halofuginon), hinolons (dekokvinat, metilbenzakvat), sulphonamides (sulphakvinoksalin), symmetric triazinons (toltrazuril) and tiamine antagonists (amprolium).

With the exeption of ionophors, there is a possibility that coccidias develop resistance (*Jordan, 1990; Dimitrijević et al., 1992; 1998*). It is required only that several sporozoits survive and start the asexual cycle. That leads to production of several thousands of parasites that are resistant to a particular drug. In order to avoid resistance, it is better to use coccidiocides that act onthe late stages of shizogony (*Jezdimirović, 1997*).

In the aim to minimize the possibility for resistance to develop, it is possible to use ”shuttle” and ”dual” program. The basis of such program is to change drugs during flock raising. Another program is the ”switch” program i.e. changing the drug for the next flock. Whatever drug is in use, it is essential to change drugs according to the mode of action of the active substance. Only in that case there is a real chance to avoid development of resistance within the parasite population (*Calnek, 1997; Dimitrijević and Ilić, 2003; Dimitrijević and Ilić, 2011*).

After treatment, whether prophylactic or in therapy, there is need to take care of drug withdrawal period. Nowadays, in order to prevent the disease, most often iodophors are in use. Drugs are omitted in the feed for the final fattening period. Nevertheless, even witho ionophores there is a possibility for the parasite to develop resistance (Chapman, 1997).

Immunity

Immunity in broilers which survived caecal coccidiosis, is life lasting and that is normal in natural infeciton (*Pellerdy, 1974*). Chicken, acquire immunity from their mothers only if hens are actively immunized against coccidiosis (*Hammong and Long, 1973*). Level of immunity depends on the age (*Ruff, 1991*) and genetic background (*Jeffers and Shirley, 1982*). At the same time, it depends on the number of oocysts that are innoculated. Immunity against coccidiosis is highly specific and cross protection has not been documented. That means that different species of the parasite can cause disease in susceptible birds (*Hofstad, 1984; Ilić et al., 2003a*).

Early informations on immunity against coccidiosis show that in order to stimulate the immune reaction, it is required to have, as immunigen, shizonts of the second generation. However, it has been shown that the immune reaction develops as early as 72 (*Kendall and McCullogh, 1952*) hours after ingestion or after intracutaneous injection (*Pellerdy, 1974*), of the infective oocysts at the time when there are not second generation of the shizonts developed jet.

Good protection in the case of coccidiosis means that there is no development of the parasites and onset of oocysts during reinfection. That is achieved after several natural infections. Better protection is achieved with every day infection of chickens with a small number of infective oocysts in comparison with one single dose (Joyner and Notrhon, 1973). In practice, simulation of multiple dose immunization is during floor husbandry when continous reinfection keep the immune system in contact with the immunogen (*Šibalić and Cvetković, 1996; Jordan, 1990; Dimitrijević and Ilić, 2011*).

The immune response to coccidia is complex. Animals infected with Eimeria spp. develop parasite-specific immunoglobulins that are present in the circulation, as well as on the mucous membranes, in secretions. However, it has been shown that specific antibodies play a minor role in the protection against coccidiosis. Nowdays there is evidence that cell imunity plays a major role in the protection against infection (*Challey and Burns, 1959; Pattillo, 1959; Daviesandsar., 1963; Soulsby, 1972; Lillehoj and Trout, 1996; Ilić et al., 2003a, 2003b*).

Early investigations show that the basis for protection against coccidiosis are of the humoral type (*McDermot and Stauber, 1954; Itagaki and Tsubokura, 1955*). However, today it has been shown that the protection is of the cellular type (*Long and Pierce, 1963*). Details of the protective mechanisms that are activated during infection are not clarified jet however, it is clear that cellular immunity plays the most important role in bird protection (*Lillehoj and Bacon, 1991*).

As a result of infection, T lymphocites produce cytokines. At the same time, T lymphocites are cytotoxic to infected cells (*Lillehoj and Trout, 1996*). However, detailed mechanisms of that protection are still obscure. One of the theory is that the major mechanism of protection is the presence of intestinal immune system of chickens, that means that the intestinal lymphoid tissue poses as the first specialized line of defence of the mucous surfaces. That system encirculates not only immunoregulatory, but effector cells, as well.

Vaccination

Resistance against anticoccidials develops very often and because of that, vaccination is the most appropriate method for desease control (*Augustine et al., 2001*). Vaccination is the simpliest and cheapest way to achieve immunoprophilaxis. In that way, the immune system is activated so natural

infection causes a secondary immune reaction which is faster and better in comparison to the primary immune reaction (*Naglić and Hajsig, 1993; Dimitrijevi and Ilić, 2003a*).

Using the ideal vaccine, the long lasting immunity is stimulated and it has to be not only specific for the basic pathogenic coccidia species, but also against strains that develop during epizootia (*Dimitrijević, 1993*). The vaccine also has to be harmless for birds that are vaccinated. At the same time the vaccine must not contaminate the natural habitat with potentially pathogenic coccidia. Vaccines that are in use, can have atenuated (alive), recombinant or antiidiotypic immunogens. As immunogens, atenuated vaccine can have non-virulent coccidia strains or can be produced on the basis of virulent coccidia strains (*Lillehoj and Trout, 1993*).

Virulent coccidia strains are used as live vaccine and consisted of a mixture of all viruelnt species and it is mostly used in drinking water (*Jordan, 1990*). They elict the most potent immune reaction since immunogenic characteristics match with the ability of the parasite to replicate and with the level of pathogenicity (*Naglić and Hajsig, 1993*). They are the best vaccines however, such vaccines have to be used in small doses in order pathogenic changes not to occur (*Orlić et al., 1996; Dimitrijević, 1997*). For maximal effect, birds have to be revaccinated several times (*Orlić et al., 1996; Dimitrijević, 1997*). Special advantages of live vaccines is that vaccine strains compete with natural, highly virulent strains that are resistant to drugs (*Hofstad, 1984*).

Recently, as the immunogen in vaccines, there are alive *Eimeria* species that are tollerant to iodophores. Advantage of such vaccines is that in vaccinated flock iodophores can be used in the first 3-4 weeks of bird life, at the time when immunity is not jet fully developed (*Danforth, 2000; Dimitrijević and Ilić, 2011*). Vaccinated birds, for not jet clear reasons, have a smaller mortality in comparison untreated ones (*Williams, 2002*). Live, virulent immunogens (vaccines) are not quite appropriate for broilers since there is a possibility of accumulation of parasites in the floor (*Lillehoj and Trout, 1993*).

Live attenuated vaccines can be divided into two groups. The first group comprises of vaccines that are made of natural strains that are of low virulence. The second group of such vaccines, have laboratory produced low virulence strains as immunogens (*Shirley, 1989*). By attenuation of infectious oocysts, live cycle of coccidia can be shortened in order to enable required number of immunizing stages and still not possessing an infectious potential (*Dimitrijević, 1993*).

Advantages of attenuated vaccines, in comparison to virulent vaccines are that in the production of a great number of oocysts, there is minimal danger of infection to occur. The disadvantage is that there is only a partial protection against natural „field“ coccidia strains (*Shirley, 1989; Augustine et al., 1993*).

Vaccines based on recombinant techniques consist of immunogens that were produced in bacterial vectors. In that way, large quantities of immunogen can be produced (*Dimitrijević, 1997*). They are a kind of cocktail consisting of different antigens originated from several coccidia species. At the same time, such vaccines consist of different antigens from the same coccidia species. To produce them, it is required to use complex technology and their production is still a matter of future in vaccinology. Disadvantages of such vaccines are low immunogenicity and possible selection of mutant coccidias that do not possess the cloned gene. So, such mutant parasite can freely replicate in the vaccinated bird population. At the beggining, in few parasite generations, mutants represent a small population however, during epizootia, they became dominant. That means that in such a case, there is a need to produce new recombinant immunogens frequently (*Lillehoj and Trout, 1993; Dimitrijević and Ilić, 2005*).

Anti-idiotypic vaccines are a special variety of vaccines that use anti-idiotypic immunoglobulins (*Lillehoj and Trout, 1993*). The mode of action of such antibodies is based on idiotypic-antiidiotypic network. Anti-idiotypic vaccines open new possibilities in coccidiosis immunoprophilaxis however, they are very expensive. At the same time they lack immunogenicity (*Naglić and Hajsig, 1993*). In future, such immunization could be used for overcoming certain genetical limitations that are still causing problems in vaccination against some other diseases (*Lillehoj and Trout, 1993*).

Chicken meat is commonly used in the each national kitchens around the world due to that there is not religious, cultural and other negative aspects of consumption. According to that, broiler production raises permanently in the farms with very big chicken population, where the possibility of appearance of coccidiosis raises too (*Dimitrijević and Ilić, 2005; Lilić et al., 2009; Dimitrijević and Ilić, 2011*). In these conditions it is very difficult to satisfied good fattening performances as well healthy animals. Fattening performances and meat quality of chicken meat depends on the many factors as provenience (*Lilić et al., 2010a; Lilić et. al, 2011a; Lilić et al., 2011b*), feeding (*Sahraei, 2012*), nutrients in the feed mixtures (*Lukić et al., 2012; Milić et al., 2012*) and the presence of probiotics in the feed mixtures (*Ivanović et al., 2009*). Nowadays, the fatty acid composition of chicken meat is permanently investigated (*Bedeković et al., 2012*). Except the influence on the fattening performances, coccidiosis causes smaller carcass yield and meat quality (*Lilić, 2007*), as well less water binding capacity (*Lilić et al., 2010b*). According to mentioned, it is very important to find the best way to protect chicken of coccidiosis because of health conditions of flock, huge economical loses due to bad fattening performances as well as bad eating and technological quality of chicken meat.

Značaj kokcidioze u proizvodnji živine

S. Lilić, S. Dimitrijević, T. Ilić

Rezime

Kokcidioza predstavlja stalan zdravstveni problem u živinarstvu širom sveta, naročito u intenzivnim sistemima gajenja živine sa velikim brojem životinja. To je najvažnije parazitsko oboljenje, za čiju se profilaksu i lečenje, troši preko dve milijarde eura godišnje. U Srbiji, najviša prevalenca je kod pilića, nešto manje kod ćuraka, gusaka, pataka i fazana. Incidenca izbijanja bolesti zavisi od raspoloživog prostora na farmi, visokih temperatura i visoke relativne vlažnosti, zatim od neadekvatne ishrane, nekih drugih bolesti i svih faktora koji dovodi do kompromitovanja imunološkog sistema životinja i opšte rezistencije prema infektivnim oboljenjima. Uzročnik infekcije su protozoe koje pripadaju familiji *Eimeridae*, pri čemu sporulisane oociste predstavljaju infektivni oblik. Izvor infekcije su inficirane ptice, kada se bolest širi u populaciji direktnim kontaktom i indirektno preko prašine, objekata na farmi, ljudi, glodara, divljih ptica i insekata. Bolest se najčešće javlja u proleće i jesen, odnosno u vlažnim sezonama sa mnogo kiše. Parazit se razvija u epitelijalnim ćelijama creva svih vrsta ptica, kao i u epitelijalnim ćelijama bubrežnih glomerula kod gusaka, u kojima se dešava razvoj merozotia i šizonata (razvojni oblici parazita) koji uzrokuju lezije i deskvamaciju sluznice. Kliničku sliku oboljenja odlikuje poremećaj opšteg zdravstvenog stanja sa dijarejom koja se tretira kao karakterističan simptom. Dijagnoza se zasniva na kliničkim simptomima, patomorfološkom i patohistološkom nalazu, kao i mikroskopskim pregledom uzoraka fecesa. Za kontrolu kokcidioze živine koriste se mere profilakse i to opšte mere profilakse: mehaničko čišćenje, pranje i dezinfekcija; takođe i korišćenje vakcina i dodavanje antikokcidijala u kompletne smeše za ishranu (kokcidiostatici i kokcidiocidi). Ekonomske posledice kokcidioze živine su: loša konverzija hrane, smanjeni dnevni prirast, smanjena telesna masa na kraju tova, produženo trajanje tova i troškovi lečenja. Prirast mase je smanjen, kao i akumulacija masti u abdominalnom masnom tkivu. Boles ima negativan uticaj na hemijske i senzorske karakteristike mesa. Jedan od problema vezanih za kokcidiozu je i rezistencija prema lekovima. U današnje vreme, kontrolne strategije zasnovane su na „shuttle“ i „switch“ programu profilaktičke medikacije, dobre proizvođačke prakse i pravilne sanitacije objekata.

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EFFECT OF EDIBLE COATING AND STORAGE TEMPERATURE ON THE QUALITY OF TABLE EGGS

M. Kenawi[1], M. P. Petrović[2], V. Caro Petrović[2]

[1] Dept. Food Sci., Minia University, Egypt.,
[2] Institute for Animal Husbandry, Belgrade-Zemun, Serbia.
Corresponding author: mohamedkenawi@ymail.com, mohamedkenawi@rocketmail.com
Invited paper

Abstract: Table eggs were coated with chitosan and stored for ten weeks at two different temperatures (4 °C and 35 °C) in order to study the double effect of edible coating and storage temperature on their quality. The data showed an increase in % of weigh loss for the uncoated samples (10% and 18%) stored at (4 °C and 35 °C) respectively. The albumen pH value increased from (pH 8.8 to 9.6), and from (pH 8.75 to 10.4) for the control samples stored at (4 °C and 35 °C) respectively. The yolk index values gradually decreased with increased storage period. The effect of chitosan edible coating in preserving the egg yolk quality was more pronounced at 35 °C. The data indicate that the storage temperature and the application of edible coating, had effect on the foam volume of table eggs during storage. It is suspected that the pH increase is responsible for the increased foam volume.

Introduction

Eggs are an inexpensive source of high quality proteins and other nutrients *(Rocculi et al., 2009)*. They are consumed worldwide as food and used as functional components of other food products. Therefore, their production has represented an important segment of the world food industry *(Torrico et al., 2011 and Wardy et al., 2011)*. Egg albumen is used in many food formulations as a foaming ingredient. The foaming properties of a foaming material are evaluated by its foaming capacity, foam stability and foam viscoelasticities. The foaming properties, in return, determine the use of the foaming material in a food product. Improving the foaming properties of egg albumens could substantially expand its use in a variety of food products *(Liang and Kristinsson, 2007)*.

Eggs are highly perishable and can rapidly lose their internal quality due to the loss of moisture and carbon dioxide through pores of the eggshell as a result of improper storage methods. Interior quality deterioration and microbial contamination of eggs during storage cause serious economic loss to the poultry

industry. During storage, loss of moisture and carbon dioxide via the shell pores causes negative quality changes in albumen and yolk as well as weight loss of eggs *(Stadelman, 1995)*. Egg preservation relies mainly on time-temperature control, carbon dioxide atmosphere packaging, controlled humidity and the use of edible coatings on the shell. In some developing countries of the world, refrigeration of eggs may be seldom practiced, and coating of eggs is an alternative and effective method to preserve their internal quality.

Coating materials including mineral oil *(Torrico et al., 2011)* and chitosan *(Kim et al., 2008)* have been applied on the surface of eggs to preserve their internal quality. Despite the fact that chitosan films are efficient barriers against permeation of oxygen, they act as low water barriers due to their strong hydrophilic properties *(Butler et al., 1996)*. Although edible films perform less efficiency than their synthetic counterparts in terms of prolonging the shelf-life of food products, they maintain an advantage of being biodegradable *(Wardy et al., 2011)*.

Several factors affecting internal quality of eggs during storage include initial egg quality and storage conditions *(Torrico et al., 2011)*. The most profound factor that affects quality deterioration rate of eggs is storage temperature *(Wardy et al 2010)*. Shell coatings and refrigerated storage provide adequate means of egg preservation although coating alone has been found to be effective during short-term storage *(Caner 2005 and Wardy et al 2010)*.

The main objective of this investigation is to study the double effect of edible coating and storage temperature on the quality of table eggs stored for ten weeks.

Key words: Table egg, edible coating, storage, quality.

Materials and methods

Materials: Fresh white shell eggs were obtained locally, washed with tap water and fan air dried then kept at approximately 5 °C immediately after purchased.

Preparation of coating solution: For preparing the coating solution, 3% chitosan was dissolved in a 1% acetic acid solution with addition of 25% glycerol (w/w, chitosan) in the mixture *(Park et al., 2004)*. Eggs were coated by dipping into the coating solution for 30 sec. followed by air drying on a stainless steel screen. A second coating was applied by dipping the eggs for 10 sec. in the same coating solution, followed by the previously described drying procedures. The eggs were then kept at two different temperatures 5 °C and 35 ° C for 10 weeks as described in Fig. 1.

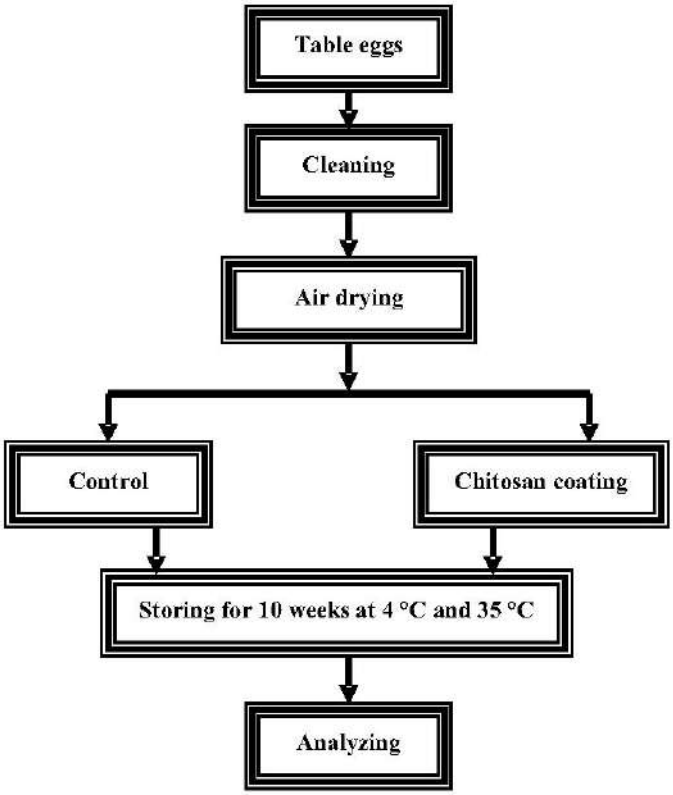

Fig. 1. Flow diagram of edible coating and storing table eggs.

Determination of weight loss: Weight loss (%) of the coated whole egg during storage was calculated as {[initial whole egg weight (g) after coating at day 0 – whole egg weight (g) after storage]/initial whole egg weight (g) after coating at day 0} × 100. Weight loss (%) of the control (uncoated) whole egg was calculated as {[initial whole egg weight (g) at day 0 – whole egg weight (g) after storage]/initial whole egg weight (g) at day 0} × 100. The weight of whole eggs was measured with a balance. Two replicates (5 eggs/replicate) per each treatment (10 eggs total/treatment) were taken *(Torrico et al., 2011)*.

Determination of yolk index, and albumen pH: Yolk index was calculated as yolk height/yolk width *(Stadelman 1995; Lee et al., 1996)*. After measurement of yolk index, the albumen was separated from the yolk. Both thin and thick albumen were mixed thoroughly prior to measuring pH with a pH meter (IQ150, IQ Scientific Instruments, San Diego, Calif., U.S.A.). Two replicates (5 eggs/replicate) per each treatment (10 eggs total/treatment) were taken.

Determination of foam volume: Foam volume was measured using a modified method by *(*Silversides *and Budgell 2004)*. Approximately 60 g of albumen from 2 eggs for each treatment were used in foam testing. Mixing for two minutes was done using a Kitchen Aid K45SS model (Kitchen Aid Company, St. Joseph, MI). Foam was measured in milliliters of foam per gram of albumen.

Results and discussion

Eggs are an excellent source of protein and certain vitamins and minerals. However, several problems such as weight loss and interior quality deterioration are encountered during storage of eggs, causing a major economic loss to the poultry industry (*No et al., 2005*). The weight loss of eggs during storage is mainly caused by evaporation of water and loss of CO2 (*Caner, 2005*). Figure 2. Illustrated are the % weight loss behavior of uncoated (control) and edible coated egg samples stored at two different temperatures (4 °C and 35 °C). With the passing of storage time, the uncoated (control) samples showed increasing levels of percentage weight loss, reaching values of about 10% and 18% for samples stored at 4 °C and 35°C respectively, at the end of a storage period of 10 weeks. On the other hand, the level of percentage weight loss for the edible coated samples reached values of about 1% and 4% for samples stored at 4 °C and 35°C for the same period. *(Bhale et al., 2003*) reported that the differences in weight loss between samples may be due to the storage conditions, temperature, egg size and shell porosity.

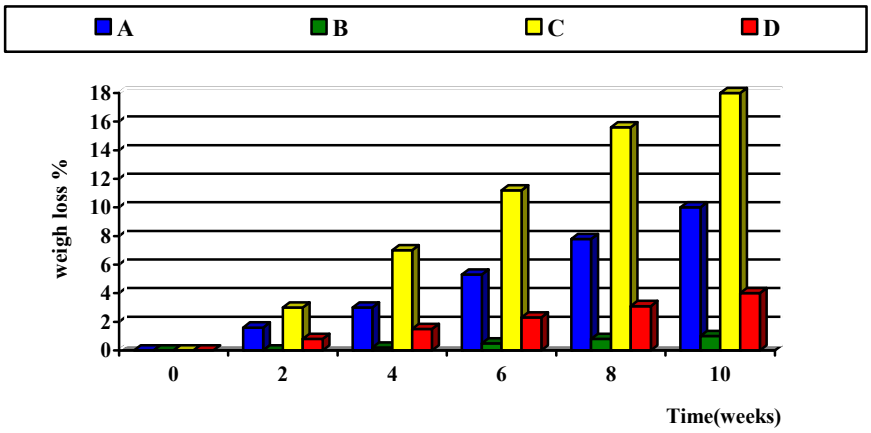

Fig. 2. Effect of edible coating and storage temperature on the weigh loss of table eggs. A = Control at 4 °C. B = Edible coated at 4 °C. C = Control at 35 °C. D = Edible coated at 35 °C.

In terms of pH (Fig.3), the albumen of uncoated (control) samples showed an increasing trend from the beginning to the end of the storage period caused by CO2 loss through the shell *(Keener et al., 2001)*. Starting from fresh eggs with an average pH value of 8.8, the albumen of the control samples reached values of 9.6 and 10.4 after 10 weeks at 4 °C and 35 °C respectively, while the albumen pH values for edible coated samples started at the same two temperatures and the same period were 8.9 and 9.36 respectively.

Yolk index is used to evaluate the degree of freshness of eggs. A decrease in the yolk index value during storage indicates a progressive weakening of the vitelline membranes and liquefaction of the yolk caused mainly by diffusion of water from the albumen (*Obanu & Mpieri, 1984*). Changes in yolk index values of the control and edible coated eggs during the 10 weeks of storage at 4 °C and 35 °C were shown in Fig.4. Overall, the yolk index values gradually decreased with increased storage period. The effect of chitosan edible coating in preserving the egg yolk quality was more pronounced at 35 °C, where the yolk index value (0.35) of the edible coated eggs was higher than that (0.17) of the uncoated (control) eggs after 10 weeks of storage. At 4 °C, no big difference in the yolk index values between uncoated and chitosan coated eggs was observed until the end of the10 weeks storage period. Cold storage (4 °C) also inserted a significant effect in preserving the yolk quality of table eggs.

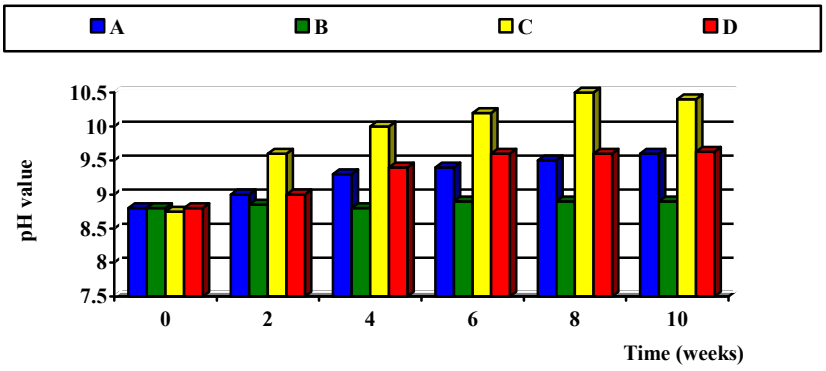

Fig. 3. Effect of edible coating and storage temperature on the pH value of table eggs. A = Control at 4 °C. B = Edible coated at 4 °C. C = Control at 35 °C. D = Edible coated at 35 °C

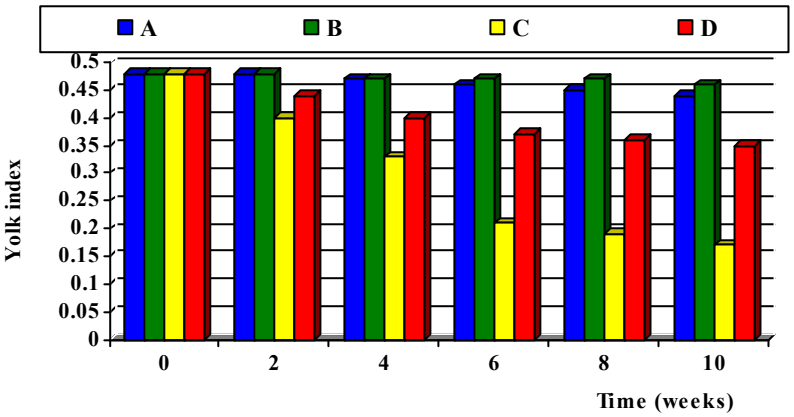

Fig. 4. Effect of edible coating and storage temperature on the yolk index of table eggs. A = Control at 4 °C. B = Edible coated at 4 °C. C = Control at 35 °C. D = Edible coated at 35 °C

Functional properties of foods determine the application and use of such food materials as ingredients for the production of various food products. Egg foam volume is very important in manufactured egg products. The foam volume results of control and edible coated table eggs stored for ten weeks at two temperatures (4 °C and 35 °C) are shown in (Fig. 5). The data indicate that the storage temperature and the application of edible coating had an effect on the foam volume of table eggs during storage. The data showed an increase in the foam volume values for the uncoated (control) table eggs. The increment was from (8.15 to 9.09 mL/g of albumen) and from (8.2 to 9.85 mL/g of albumen) during the ten weeks of storage at 4 °C and 35 °C respectively. On the other hand, the increment for the edible coated table eggs was from (8.1 to 8.85 mL/g of albumen) for the eggs stored at 35 °C.

It is suspected that the pH increase is responsible for the increased foam volume. *Bovskova and Mikova (2011)* found that, the worst whipping quality of non-pasteurised and pasteurised egg white was observed at pH 5 and in the pH 7.5 which corresponds to common pH of egg white.

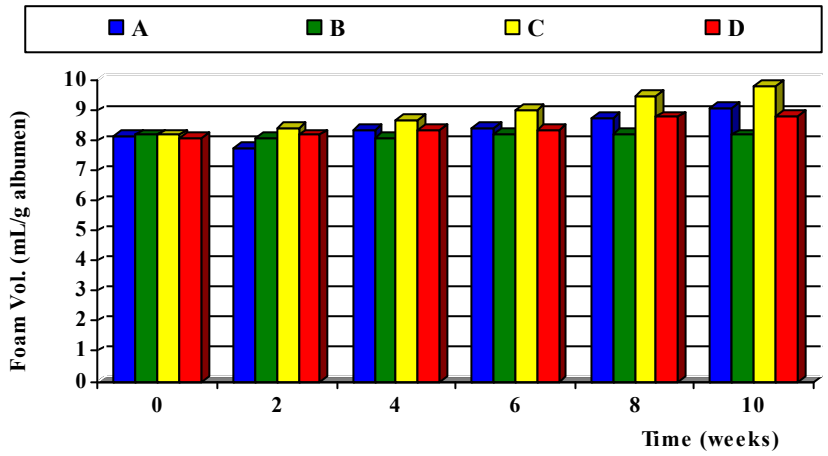

Fig. 5. Effect of edible coating and storage temperature on the foam volume (mL/g of albumen) of table eggs.
A = Control at 4 °C. B = Edible coated at 4 °C. C = Control at 35 °C. D = Edible coated at 35 °C

Conclusion

Table eggs coated with edible coating (chitosan) and stored for ten weeks at 4 °C, have less weight loss, lower pH values and higher yolk index values than the uncoated or the ones stored at higher temperature (35 °C).

Uticaj jestivih prevlaka i temperature skladištenja na kvalitet konzumnih jaja

M. Kenawi, M.P. Petrović, V. Caro Petrović

Rezime

Jaja su obložena hitosanom i skladištena u periodu od deset nedelja na dve različite temperature (4 ° C i 35 ° C), kako bi se proučio dvostruki efekat jestivog premaza i temperature skladištenja na njihov kvalitet. Podaci su pokazali povećanje % gubitka težine kod nepremazanih uzoraka (10% i 18%) koji su uskladišteni na (4 ° C i 35 ° C). pH vrednost belanca porasla je sa (pH 8,8 do 9,6), i od (pH 8,75 na 10,4) za kontrolne uzorke skladištene na (4 ° C i 35 ° C). Vrednosti indeksa

žumanca se postepeno smanjuje sa povećanjem perioda skladištenja.Efekat hitosana - jestivog premaza na očuvanje kvaliteta žumanca je bio više izražen na temperaturi 35 ° C. Podaci ukazuju da su temperatura skladištenja i primena jestivog premaza, imali uticaj na zapreminu pene konzumnih jaja tokom skladištenja. Sumnja se da je povećanje pH vrednosti odgovorno za povećanje zapremine pene.

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IMPROVING OF PRODUCTIVE AND REPRODUCTIVE PERFORMANCES IN RABBITS BY BIOLOGICALLY ACTIVE FEED ADDITIVES

D. Abadjieva[1], M. Chervenkov[1], K. Shumkov[1], A. Shimkus [2], A. Shimkiene[2], E. Kistanova[1]

[1]Institute of biology and immunology of reproduction, BAS, Sofia, Bulgaria
[2]Lithuanian University of Health Sciences, Kaunas, Lithuania
[*] Corresponding author: kistanova@gmail.com
Invited paper

Abstract: Production of ecologically clean animal products supposes the use of natural feed additives for stimulation of growth rate as well as for stimulation of reproductive potential. The most appropriate for this purpose are plant extracts and microalgae. They are a protein source and content many vitality important compounds: vitamins, minerals and phytochemicals with antioxidant and hormone stimulating activity. Due to high quality and healthfulness of rabbits' meat the special attention should be pay for rearing of these animals under ecological conditions. The aim of this investigation was studying the effect of plant extract Tribulus terrestris and dry biomass of microalgae *Spirulina platensis* added to the main diet of rabbits on their physiological and reproductive status. Two experiments were conducted with white New Zealand rabbits. In first experiment were involved 28 female rabbits at 40 days age divided in 4 groups: control and 3 experimental. Experimental animals obtained the different doses of the Bulgarian *Tribulus terrestris* L.: 2,5mg, 5 mg, 10 mg/ kg of live weight during 42 days.The live weight was controlled during the whole experimental period. At the end of treatment the routine histological estimation of the ovaries included follicular dynamic, number of follicles and their size in the serial 5 μm section of whole ovary was done. In second experiment were involved 16 rabbits of both sexes divided in two groups: control and experimental. The experimental animals received additionally the dry biomass of *Spirulina platensis* in dose 1g/per animal during 120 days before coitus. The live weight and morphological and biochemical parameters of blood was measured at the beginning and at the end of experiment. All does were covered with two control rabbits. The live born offspring were registered. In both experiments the standard diet of food and water was supplied ad libitum. The investigated feed additives had shown different biological effect on the rabbits. *Tribulus terrestris* extract affected directly the ovarian activity and changed the folliclegeneses process in dose dependent manner. The highest ovarian

activity without pathological changes was observed after treatment with dose 2,5 mg/ kg of *Tribulus terrestris* extract. *Spirulina platensis* improved the physiological state of animals (enhance the hemoglobin level, decrease the cholesterol), but not affect directly the fertility of rabbits in first generation.

Key words: rabbits, reproduction, *Spirulina platensis*, *Tribulus terrestris*

Introduction

Importance of properly nutrition for healthy and reproductive status of human and animals is undiscussable. The rabbit meat is known as an appropriate product for human diet, having higher protein content and lower fat, sodium and cholesterol contents than meat of other mammal animals (*Cobos et al., 1993, cit. by Barbosa et al., 2004*). Due to high quality and healthfulness of rabbits' meat the special attention should be pay for rearing and feeding of these animals under ecological conditions (*Dimitrova et al., 2008*).

Production of ecologically clean animal products can be achieved by use of natural feed additives for stimulation of growth rate as well as for stimulation of reproductive properties. The most appropriate for this purpose are plant extracts and microalgae. They are a protein source and content many vitality important compounds: vitamins, minerals and phytochemicals with antioxidant and hormone stimulating activity.

One of famous blue-green algae used in the human and animal nutrition in the last few years is *Spirulina platensis*. Spirulina is 60-70% protein by weight and contains a rich source of vitamins, especially, vitamin B12 and provitamin A (β-carotene), polysaccharides (calcium spirulan, immulan), and minerals (Na, K, Ca,Fe, Mn and Se) (*Belay, 2002*). Its immunomodulation, anticancer and antinflamatory effects are well known (*Kumar et al., 2010, 2009; Dembitsky et al., 2006*). Spirulina play an important role in control of hyperlipidemia and cholesterol level (*Kaur et al., 2009*) and prevents against heavy metal intoxications (*Doshi et al., 2009; Karaca and Simsek, 2007*). Positive effect of Spirulina platensis on the physiological status and productivity of agricultural animals- pigs, lambs, cows, was reported by *Shimkus et al. (2008a; 2008b), Shimkiene (2010),* and *Heidarpour et al.(2001*). Due to decreasing the cholesterol level in rabbits fed with a hypercholesterolemic diet (*Colla et al 2009*) and effect on the meat quality and fatty acid composition, *Spirulina platensis* potentially could be used in rabbit nutrition with consequent benefits on the quality of rabbit meat for consumers (*Peiretti and Meineri, 2011*).

However the data about the effect of *Spirulina pl.* on female reproductive status in livestock is very scanty. In accordance with results of *Shimkus et al,*

(2009) addition of the Spirulina to the main diet of pregnant sows leads to the higher life weight of new born piglets.

The future investigations, clarified the effect of *Spirulina pl.* on the reproduction in agricultural animals, is necessary.

The herb with hormone stimulating activity is *Tribulus terrestris*. The pharmacological value of this herb is estimated by the content of a steroidial furastanol saponine as a protodioscin. The amount of protodioscin depends on the region of herb grown. According to the *Ganzera et al.,(2001)* the most higher content of the protodioscin is in the Bulgarian samples in comparison with Chinese and Indian. *Tribulus terrestris* effect on the androgenic metabolism in animal models and human is well known from ages (*Neichev and Mitev, 2005; Gauthman et al, 2008; Tomova, 1987*).

The new results, presented *by Adaay et al.(2012)* and *Esfandiari et al. (2011)*, show the effect of this plant on female reproduction in experimental mouse and rats. Additionally it is reported that *Tribulus terrestris* extract contents many biological active substances including vitamins and microelements and effects on the vitality: decreases the cholesterol and glucose levels, acts as an antimicrobial and cytotoxic substance for new formations in the organism (*Chu et al., 2003; Li et al., 2002; Bedir and Khan, 2000*).

There are few data about the application of *Tribulus terrestris* extract in females of agricultural animals. *Valchev et al. (2008)* described the positive effect of Tribulus t. on the growth rate of does. *Grigorova et al (2008)* reported about the improved eggs quality in hens treated with *Tribulus tr*.

The scanty data about the influence of mentioned above feed additives on the female reproductive parameters in livestock got to define the aim of our work - studying the changes in reproductive status of rabbits, fed the plant extract *Tribulus terrestris* and dry biomass of microalgae *Spirulina platensis*, as additives to the main diet.

Material and method

Experiments were conducted with white New Zealand rabbits in animal facilities of Institute of Animal Science - Kostinbrod and IBIR-BAS, Sofia. In first experiment were involved 28 female rabbits at 40 days age divided in 4 groups: control and 3 experimental. Experimental animals obtained the different doses of the substance Vemoherb-T (commercial name of the Bulgarian *Tribulus terrestris* L. extract, producer firm Vemo-LTD, Bulgaria): 2,5mg, 5 mg, 10 mg/ kg of live weight during 42 days. The standard diet of feed and water was supplied ad libitum. The live weight was controlled during the all experimental period. At the end of experiment the routine histological estimation of the ovaries included

follicular dynamic, number of follicles and their size in the serial 5 μm section of whole ovary was done in the Institute of Biology and Immunology, BAS.

In second experiment were involved 16 rabbits of both sexes divided at 2 months age in two groups: control and experimental. The experimental animals received additionally the dry biomass of *Spirulina platensis* (Producer- UAB MAZURO AD, Lithuania) in dose 1g/per animal during 120 days before coitus. The live weight and morphological and biochemical parameters of blood was measured at the beginning and at the end of experiment. The total level of protein, urea, cholesterol and alkaline phosphatase activity were estimated using the commercial kits in veterinary diagnostic laboratory.

The does from both groups were covered with two control rabbits. The live born offspring were registered.

Statistical analysis: The computer statistical package STATISTIKA (Stat Soft version 6.0) was used to estimate the obtained data. Differences were considered significant if $p \leq 0.05$.

Results and discussion

Effect of Tribulus terrestris

The results had shown that the highest activity of ovaries was observed in the animals treated with 2.5 mg/kg of *Tribulus terrestris* L. In their ovaries, there is complete division of well-developed follicular structures, with a predominance of preovulatory follicles (Figure1). Tribulus t. in dose of 5mg/kg suppressed the development of preovulatory follicles (8,3 ± 2,4 per slice against 19,5 ± 4,3 in the control group, $P < 0,05$). With increasing of Tribulus doses, particularly till 10 mg/kg, re-stimulating effect was observed. The number of preovulatory follicles increased (Figure 1). However, in the ovaries of these animals the atresia of developing follicles was provoked. Already at the stage of secondary follicles the altered granulose cells as well as the separate atretic bodies (Call-Exner bodies) were observed. In the preovulatory follicles Call-Exner bodies were widely presented (Figure 2). No significant difference in size of follicular structures between the groups was established.

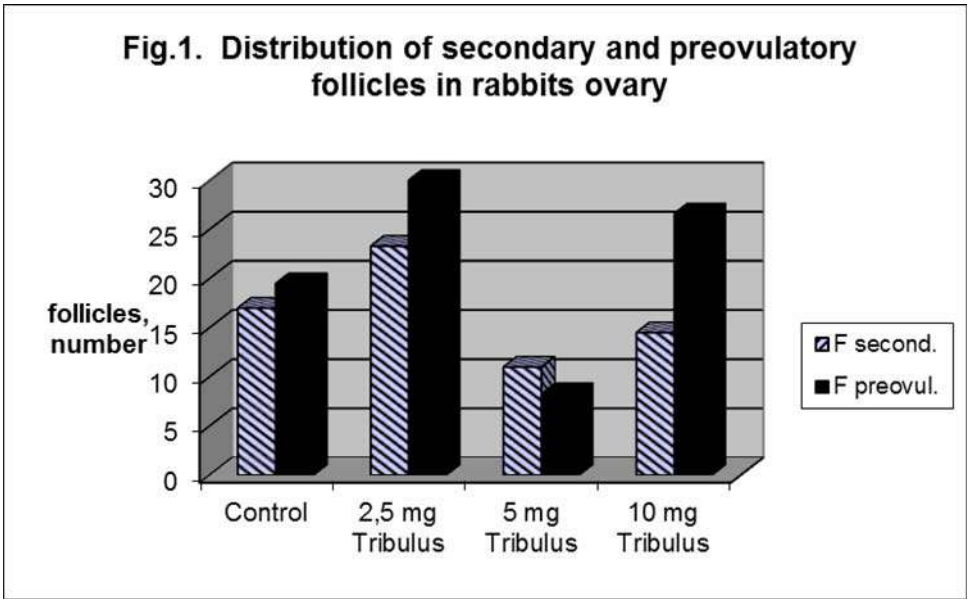

Fig.1. Distribution of secondary and preovulatory follicles in rabbit's ovary

The growth rate of rabbits, defined as the average daily gain, corresponds to the activity of the ovaries (Table 1). The maximum growth rate (13%, P <0,05 compared to control) as well as the high ovarian activity without pathological changes were observed in animals treated with dose of 2.5 mg / kg of *Tribulus tr.*

Table1. Growth intensity of female rabbits treated with Tribulus terrestris

Parameters	**Groups**			
	Control	**2,5 mg Tribestan**	**5mg Tribestan**	**10mg Tribestan**
Number of animals	7	7	7	7
Duration of treatment	42	42	42	42
Live weight - at the beginning - at the end	1078 2543	1125 2707	1046 2667	1026 2656
Daily gain, g - %	35,1±2,41 100,0	39,7±1,* 113.1	35,2±1,0 100.3	38,7±2,5 110.3

* P<0,05 compared to the control group

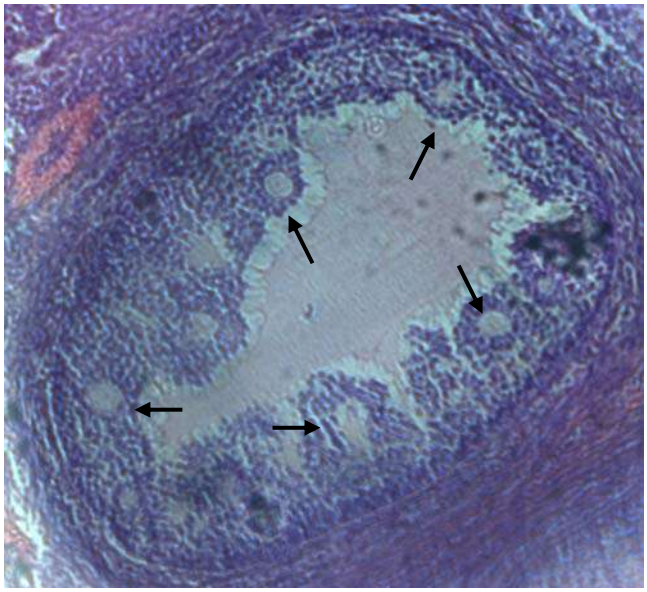

Fig.2. Preovulatory follicle with atretic (Call-Exner) bodies. Magnification 12,2X20.

Effect of Spirulina platensis

The results of Spirulina effect on growth rate of rabbits is presented in Table 2. No significant differences between average means of life weight in control and experimental groups were established. However, there is a tendency of faster intensity of growth in females.

Table 2. Growth intensity of rabbits treated with Spirulina platensis

Parameters	**Groups**			
	control females	control males	**experimental females**	experimental males
Number of animals	4	4	4	4
Duration of treatment, days	120	120	120	120
Live weight, g - at the beginning -at the end	 1960 ±140 3720±400	 1810±160 3100±250	 1540±230 3490±280	 2000±170 3350±230
Average daily gain, g - %	14,6±0,4 100,0	10,8±0,7 100	16,3±1,5 111,6	11,3±1,0 104,6

The rabbits treated with Spirulina had the lower level of the total cholesterol at the end of the experiment (Table 3). The indicators of metabolism intensity - the urea and alkaline phosphatase are significantly higher in the experimental females. Thus should explain the data that female animals have a higher weight gain at the end of the experiment.

Table 3. The physiological parameters of animals treated with Spirulina platensis

Parameters	**Groups**			
	control females	control males	**experimental females**	experimental males
Number of animals	4	4	4	4
T.protein,g/L	68,9±0,1	65,9±0,2	66,8±0	65,6±0
Urea, mmol/L	6,9±0,06	5,6±0,04	10,1±0,05*	7,1±0,02*
T.cholest,mmol/L	1,9±0,03	1,8±0,01	1,3±0,03*	0,97±0,01*
Aph, U/l	66±2,5	77,3±8,3	90±2,1*	62,3±3,7

*P<0,05 compared to the control groups

The results in Table 4 are reflected the data about the fertility of rabbits treated with *Spirulina pl*. It was not obtained the significant difference compared to the control animals.

Table 4. Fertility of rabbits treated with Spirulina platensis

N of animals **N of offspring**	**1**	**2**	**3**	**4**	**Total number offspring**	**Average/ per doe**
control	6	7	9	8	30	7,5±0,5
experimental	6	10	9	8	33	8,3±0,8

The used in the experiments feed additives shown different biological activity and effect on the rabbits. *Tribulus terrestris* extract affected directly the ovarian activity and changed the folliclegeneses process. Our results correspond with the data, obtained in the experimental mouse and rats (*Adaay et al., 2012; Esfandiari, 2011*). The authors had found the increased number of secondary and preovulatory follicles in treated with *Tribulus terrestris* animals, also they pointed out to dependence of these changes on the dose and on the duration of treatment. The supposed mechanism of *Tribulus tr.* acting is the enhancement of the LH level, which is manifested clearly in the males' animals *(Martino-Andrade et al., 2010*). *Adday et al. (2012)* reported about the tendency to increase the levels of FSH and LH in females' mouse, but it was not significant increase.

Treatment with Spirulina platensis leads to the activation of the metabolic process in the females' rabbits and to decrease a cholesterol level. This data is in

agreement with results of *Colla et al., (2009)* and *Sun Hee et al. (2010),* who underlined the hypocholesterolemic effect of Spirulina in rabbits. The similar decreased level of cholesterol was observed in calves supplemented with *Spirulina platensis (Heidarpour et al., 2011).* Despite the intensity of the metabolic processes, *Spirulina platensis* didn't affect directly the fertility in the experimental animals in first generation. The new research data show that in many cases the effect of the nutrition on reproductive parameters appears in second or third generation (*Abu-Saa and Fraser, 2010*).

Conclusion

The treatment with *Tribulus terrestris* extract provoked the changes in ovarian activity of rabbits in dose dependent manner, which correlated with the intensity of rabbit growth. The maximum of the growth intensity (13%, $P<0,05$ versus control group) as well as the highest ovarian activity without pathological changes was observed by treatment with dose 2,5 mg/ kg of *Tribulus terrestris* L extract.

Spirulina platensis improved the physiological state of animals (enhanced the hemoglobin level, decreased the cholesterol, supported high metabolic activity), but not affected significally the number of offspring in first generation.

Unapređenje produktivnih i reproduktivnih performansi zečeva korišćenjem biološki aktivnih aditiva

D. Abadjieva, M. Chervenkov, K. Shumkov, A. Shimkus, A. Shimkiene, E. Kistanova

Rezime

Proizvodnja ekološki čistih proizvoda životinjskog porekla pretpostavlja korišćenje prirodnih aditiva za podsticanje rasta kao i za stimulaciju reproduktivnog potencijala. Najpogodniji za ovu svrhu su biljni ekstrakti i mikro alge. Oni su izvor proteina i sadržaj mnogih jedinjenja važnih za vitalnost: vitamini, minerali i fito hemikalije sa antioksidantskim dejstvom kao I podsticanjem hormona. Zbog visokog kvaliteta i lekovitosti mesa zečeva, posebnu pažnju treba posvetiti uzgoju ovih životinja u ekološkim uslovima.

Cilj ovog istraživanja je bio proučavanje uticaja biljnih ekstrakta *Tribulus terrestris* i suve biomase mikroalgi *Spirulina platensis* kao aditiva u ishrani kunića na njihov fiziološki i reproduktivni status.

Dva eksperimenta su izvedena sa belim novozelandskim kunićima. U prvom eksperimentu su učestvovale 28 ženke 40 dana starosti podeljene u 4 grupe: kontrola i 3. Eksperimentalne grupe. Eksperimentalne životinje dobijaju različite doze bugarske *Tribulus terrestris L*.: 2,5 mg, 5 mg, 10 mg/kg žive mase tokom 42 dana. Živa masa je kontrolisana tokom celog eksperimentalnog perioda. Na kraju tretmana rutinska histološka analiza jajnika uključivala je dinamiku folikula, broj folikula i njihovu veličina u serijskom 5 μm delu celog jajnika.

U drugom eksperimentu, 16 zečeva oba pola podeljena su u dve grupe: kontrola i eksperimentalna grupa. Eksperimentalne životinje su dobile dodatno suvu biomasu *Spirulina platensis* u dozi 1g po životinji, 120 dana pre koitusa. Živa masa, morfološki i biohemijski parametri krvi su mereni na početku i na kraju eksperimenta. Živi potomci su registrovani na rođenju. U oba eksperimenta je bila standardna ishrana hrane i napajanje *ad libitum.*

Ispitivani aditivi su pokazali drugačiji biološki efekat na zečevima. *Tribulus Terrestris* ekstrakt utiče direktno na aktivnost jajnika i promeniće proces geneze folikula na način zavisan od korišćene doze. Najveća aktivnost jajnika bez patoloških promena je registrovana posle tretmana sa dozom 2,5 mg/kg *Tribulus Terrestris* ekstrakta. *Spirulina platensis* poboljšao fiziološko stanje životinja (podizanje nivoa hemoglobina, smanjenje holesterola), ali ne utiče direktno na plodnost zečeva u prvoj generaciji.

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BREEDS OF CHOICE IN ORGANIC PRODUCTION SYSTEM

M. Savić[1], S. Aleksić[2], D. Živković[3]

[1]Faculty of Veterinary medicine, Belgrade, Republic of Serbia
[2]Institute for Animal Husbandry, Belgrade-Zemun, Republic of Serbia
[3]The Christ College, Cincinnati, Ohio, USA
Corresponding author: mij@beotel.net
Invited paper

Abstract: The demand for organic ruminant production has increased significantly annually over the past two decades. There are multiple challenges dealing with organic ruminant production, and the most important tasks include determination of the preferable breed and health management. The paper presents an overview of criteria for selecting breeds best suited to organic dairy, beef and sheep production systems. Organic livestock production has a very important role in the Rural Development Strategy of Serbia. The monitoring of ecological and natural resources that provide optimal conditions for organic ruminant production have been performed. The importance of incorporating autochthonous breeds into organic milk and meat production systems is particularly emphasized. Additionally, a global overview of organic ruminant production is given, along with an assessment of opportunities for implementing organic ruminant production systems in Serbia.

Key words: organic farming, autochthonous breeds, health management

Introduction

At the beginning of the 21st century the question of agricultural production in the world, its current status, possibilities for development and challenges it is faced with are considered very important topics. This global, multi-disciplinary problem requires a broad approach and well-funded support of the whole scientific community. Therefore, the global conference dedicated to the strategy of "Feeding the World in 2050"(FAO) primarily emphasized the need to produce more food in rural areas, to support the development of bioenergy and to thoroughly apply the program for increasing the efficiency of self-sustaining agricultural systems. This conference also accented the importance of the awareness of global climate changes and their effect on agricultural production, as well as on agrobiodiversity. Organic and sustainable agriculture plays an important role in offering solutions to

meet these challenges (*Elliset al.,* 2012). People themselves are also raising awareness about the return to nature and healthy lifestyles through a growing interest in organic animal products. So in advertising campaigns the world's largest corporations that produce organic animal products increasingly point to products derived from animals reared in accordance with the highest standards of welfare and protection of ecosystems. In this sense, research shows that the consumption of organic products has changed the opinion of the consumer; through various activities they have to a greater extent becomes involved in the protection of ecosystems.

The high demand for organic production is recognized and reported in many regions. Analyses by continents show that the largest land area for organic agricultural production are found in Australia/Oceania, followed by Europe and America. The development of organic production in Asia is also reported, especially in China. Organic production in European countries is increasing. Results of a survey Production and Commercialization of Organic Milk in Europe (2012) shows that the most important producer of organic milk is Germany with a quantity of almost 600000 t followed by Denmark (478000 t) and Austria (431000 t). The share of organic milk deliveries (total quantity of milk with and without price markup for organic products) was the highest in Austria amounting to 15.5 % of milk deliveries. In Denmark and Sweden a share of about 10 % of the delivered milk was processed according to organic standards. The study emphasizes that the price of organic milk plays an important role for the decision of farmers to convert to organic farming.

Organic agricultural production has a very important role in the Rural Development Strategy of Serbia. Law on Organic Production is in accordance with Council Regulation (EC) No 2092/91. According to estimates, Serbia has natural resources with great potential for organic production. It should be noted that 16.15% of the area is under pasture and under the protection of more than 500 sites, totaling an area of about 600000 ha, which indicates potential for the development of organic agriculture in Serbia. Although there are export opportunities for organic animal product and government is encouraging producers, the process is slowed down by difficulties in the implementation of organic standards. In this sense, when it comes to organic ruminant production, only a small number of farms have been registered by now, however a large number are in the process of conversion. Currently the largest organic dairy farm counts about 1000 cows.

Organic ruminant production

Organic ruminant production is closely linked to sustainable rural development models, which ensures that natural resources are exploited, while a

balance is maintained with biodegradable reserves in a given habitat *IFOAM* (2010), (*Pillinget al.,*2011; *Jovanović et al.,*2009, 2011).

The demand for organic ruminant production has increased significantly each year over the past two decades. Global demand for organic products like milk, sheep and goat cheeses, meat and fiber is an important opportunity for the future growth of organic markets at 10–30% annually.

In order to develop organic production process, the Faculty of Veterinary Medicine, University of Belgrade, is taking an active role on the implementation of course curriculum related to organic livestock management for undergraduate and postgraduate students and knowledge transfer to extension services.

The monitoring of ecological factors and animal genetic resources, as well as the study of the possibilities for implementation standards of organic ruminant production in some hilly mountain regions has been performed.

Previous experience in countries with developed organic livestock production show that there is a number of problems in the organization of organic production (self-sufficient farms, conversion period, the land must not have any prohibited substances, preventive measures, integrative medicine, use conventional vs. alternative modalities etc.).The most important challenges in dealing with organic ruminant production are determination of the preferable breed and health management.

Several surveys have shown that many of the breeds used in intensive conventional breeding have high production characteristics, but under organic condition they are unable to express their production potential. The study of 'genotype x environment' interactions is needed for decision making in organized and organic livestock production. In organic breeding, breeds are reared under altered condition and have specific characteristics (*Nauta,* 2009). Organic production standards emphasisis on allowing animals to adapt to local conditions and the preservation of genetic diversity. The advantage of autochthonous breedsis that they are genetically adapted to specific environmental conditions.

Breeding strategies in organic ruminant breeding is based on the classic principles: selection between breeds, crossbreeding, and selection within the breed. Traits important for organic breeding have been evaluated by scientific institutions and organic producers (*Van Diepenet al.,* 2007). The very important fact for veterinary services is that the selection program in organic ruminant production is based on traits primarily related to health, fertility and longevity. Considering the characteristics and ability for breeding under organic conditions the preferable breeds for specific environment and production systems are also recognized.

Organic dairy breeds

Results of numerous studies have found that fertility and ease of calving, general disease resistance, udder health (conformation udder, mastitis resistance),

long productive life, good milk yield/lactation, protein and fat content, forage intake capacity, feet and leg strength, susceptibility to lameness, resistance to parasite infestation, robustness/hardiness, temperament, marketability of milk and calves are very important traits for organic dairy breeding (*Klocke et al.,*2012).
Both regional and national reports have shown that different production and marketing strategies demanded different breeds (*De Haaset al.,* 2012). Farmers specializing in milk production use purebred Holstein and crosses with robust breeds, such as Brown Swiss, Monbiliarde. Good results are also reported for Friesians (FH), Jersey, Guernsey, Ayrshire etc. There are distinct benefits to using organic Holstein Jersey heifers for a organic dairy farms. Many studies emphasize the results of the dual purpose breeds for organic dairy production (*Verkampet al.,* 2012).

Mastitis and reproductive disorders are the most common health problems which occur in organic dairy production (*Brinkman et al*., 2012, *Penedoet al.,* 2012). In this sense, the selection is increasingly focused on general disease resistance and udder health. As a general problem the reports underscore the marketing problems of male calves which are an on-going difficulty for both organic and conventional producers.

Organic beef breeds

The main beef breeds widely spread throughout the world like Hereford, Limousin, Charolais, Belgian Blue are successfully used in organic farming. The meat quality of these breeds is well known. The most important traits for organic beef breeding are ease of calving, forage conversion, lameness and mastitis, suitability for conservation grazing, marketability of meat, finishing before 24-30 months. In different specific regions the local breeds, characterized by good forage conversion and suitable for conservation grazing have selective advantages in organic beef production. These are well adapted and traditional breeds (Angus, North Devon, etc.) which are good for direct marketing and promotion of the region.

Organic sheep and goat breeds

Organic sheep production is focused on the meat production. Different breeding systems and farm sizes are present. The most important sheep breed production traits are related to quick fattening. Also, election criteria consider ease of lambing, foot rot, worm resistance, and suitability for conservation grazing. Good results under organic condition are reported for Texel, Suffolk and Charolais. Some local breeds are important for conservation grazing. In Germany, as an example of rational organic production, the results of fattening Württemberg lambs on pasture and in the mixed system are recognized.

In Europe, organic goat production has the highest growth in countries that have traditionally been oriented to goat production such as Italy, Greece, France, Spain and United Kingdom (*Luaet al*., 2010). Organic goat production is based on high performance goat breeds, like Alpine (Alpine German Brown) in Germany, which have good productive results under organic conditions (*Rahmann et al.,* 2006*,* 2009**)**. With consideration of its high price, organic goat milk is a profitable product. In some areas, the Boer breed is preferable. Indigenous breeds well adapted to modost conditions as well as Payoya breed in Andalusian mountainous areas have advantages (*Acostaet al.,*2009).

One of the major health problems in sheep and goat organic production is parasite control (*Rahmannet al.,* 2006; *Taylor et al.,* 2012).

The importance of autochthonous breeds in organic farming

Generally on organic farms there was a trend towards the use of more autochthonous breeds.The study and preservation of animal genetic resources, relies on the management of a database (FAO DAD-IS sytem) that includes phenotype and genotype characteristics and other health criteria, such as reistance/tolerance within a population to a variety of pathogens. This database serves as the foundation for further research and the advanced use of genetic resistance factors to diseases. When considering the significance of resistance/tolerance at the breed level, the intrinsic evolutionalry advantage of breeds that are adapted to an environment should be taken into account *Bishop* (2002). In regions, where endemic diseases are widesdpread, due to their evolutionary roots, locally adapted autohtonous breeds display a far greater level of genetic resistance and adaptation, as compared to imported breeds.

In the scope of multidisciplinary research the possibilities for organic livestock production in the hilly mountain region were examined. The autochthonous Zackel sheep breed is traditionally reared in Balkan hilly mountain regions. Nowadays, various types of Zackel sheep are endangered according to FAO critetria. A major driver for dealing with endangered Zackel sheep has been crossing them with exotic Wurttemberg and Ile d France breeds. By recognizing that locally adapted breeds gained genetic resistance and adaptability through the evolutionary process, breeding strategies in sustainable farming practices today have become far more attuned to the need for preserving and utilizing these autochthonous breeds. Considering the importance of autochthonous breeds the uses and values of Sjenica sheep as AnGR have been evaluated *Savić et al.,* (2012). Sjenica sheep is the biggest transboundary Zackel type, traditionally reared at Sjenica-Pester plateau (900-1200 m), a wide pasture in southwestern Serbia. Although Sjenica sheep is a low productive triple-purpose breed, it is fully adapted to the challenges of the environment and harsh climate condition on the Sjenica Pester plateau. During the last winter the temperature reached at times -30^{0}C.

Considering that the development of organic agriculture contributes significantly to rural development characteristics of Sjenica's natural resources, the possibilities for organizing organic sheep production were examined.

Conclusion

The organization of organic production in Serbia's hilly mountain regions has a great impact on rural development, esspecially in regions with a significant depopulation trend. Those are areas with low input ruminant production, where there are natural resources for the development of organic livestock. Autochthonous breeds, especially Zackel breed, in many areas are well known for traditional animal products, such is Sjenica cheese. The involvement of these breeds in organic production will accentuate their value as animal resources. Organic livestock development strategies have a direct positive influence on the promotion and revival of the entire region.

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Rase pogodne za uzgoj u organskim proizvodnim sistemima

M. Savić, S. Aleksić, D. Živković

Rezime

Potražnja za mlekom i mesom preživara, dobijenim u organskim uslovima, značajno se povećava iz godine u godinu. Postoji veći broj izazova u organizovanju organske proizvodnje preživara, među kojima se izdvaja izbor rase. U radu je dat pregled rasa koje najbolje odgovaraju uzgoju u organskim sistemima proizvodnje. Organska proizvodnja ima veoma važnu ulogu u strategiji ruralnog razvoja Srbije. Preduzeta su ispitivanja prirodnih resursa koji imaju dobre uslove za organizovanje organske proizvodnje. U radu je naročito istaknut značaj autohtonih rasa u organskoj proizvodnji. Pored toga, prikazan je globalni pregled organske proizvodnje, kao i procena mogućnosti za šire organizovanje organske proizvodnje preživara u Srbiji.

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STATE AND PERSPECTIVES OF SHEEP BREEDING DEVELOPMENT IN RUSSIA

A.I. Erokhin, E.A. Karasev, I.N. Sycheva

Russian State Agrarian University named after K.A. Timiryazev, Moscow, Russia
Corresponding author: in_sychewa@mail.ru
Invited paper

Abstract: One of the most important branches of agriculture in Russia is sheep breeding. Sheep provide raw material for meat-processing and textile industries, the sheep breeding industry products are the source for the folk craft. Sheep breeds bred in Russia (39 breeds) have different productivity specializations: fine-wool sheep (14 breeds); half-fine-wool sheep (11 breeds); half-hair sheep (2 breeds) and hair sheep (2 breeds). These breeds are bred in our large country according to climatic zones. In many Russian regions where breeding of other farm animals is impossible sheep are the leading type of bred animals due to their excellent adaptive qualities. The sheep number influences directly the local population employment and often is the main source of income. In recent years due to the change of the form of property the main increase of sheep number has been registered in private peasant households. The wool production unprofitability and low purchase prices for wool result in the reduced demand for wool and increased demand for mutton. So presently the great attention is given to the development of early maturing meat and meat-wool sheep breeding. The local hair sheep as the source of meat production increase are widely bred. That is why the main tendency of sheep breeding development nowadays is the breeding of sheep combining the high meat production and crossbred wool.

Keywords: sheep breeding, sheep breeds, meat, wool, federal program of industry development

Introduction

Sheep breeding is one of important animal breeding branches in Russia *(Erohin A.I and Erohin, 2010)*. Sheep provide various types of products: wool for many purposes; mutton; fat raw material; milk; fur, wool and sheep skins; lamb skins. This variety of sheep breeding production is due to the great amount of sheep breeds bred in Russia (39 breeds) and the wide spectrum of their

specialization: fine-wool sheep of wool, wool-mutton and mutton-wool specialization (14 breeds); mutton and mutton-wool half-fine-wool sheep (11 breeds); half-hair sheep (2 breeds); hair sheep of lamb skin, mutton-fur, mutton-wool, mutton-fat and mutton-wool-milk specialization (12 breeds). Such great amount of breeds and their productivity specializations are explained by considerable variety of climatic, social and economic conditions of various Russian regions.

Special attention should be given to sheep breeding development not only because of valuable products (mutton, milk, wool, etc.) but also as a factor ensuring the employment in regions not suitable for breeding other farm animals because of unfavourable climatic conditions *(Erohin et al.,1985,2001, 2010).*

Number of sheep and production of main sheep products

By the end of 2011 the number of sheep in all types of farms in Russia was equal to 20 766.8 heads (Table 1).

Recently the growth of sheep number on various types of farms is due to peasant households.

Table 1. Sheep number on various farms in Russia

Farm type	Year				2011/ 2000, %
	2000	2007	2009	2011	
Sheep number by the end of year, thousands of heads					
Farms of all types	12730.5	19290.4	19850.5	20766.8	163
Agro organizations	4499.4	3944.8	4467.8	4268.6	95
Households of population	7448.1	9356.2	9507.5	9684.0	130
Peasant households	783.0	5989.4	5875.2	6814.2	870
% of total number					
Farms of all types	100	100	100	100	100
Agro organizations	35.3	20.4	222	20.6	- 14.7
Households of population	58.5	48.5	47.9	46.6	- 11.9
Peasant households	6.2	31.3	29.6	32.8	+26.6

In the last decade (2000-2011) the sheep number in peasant households increased in 8.7 times and in agro organizations decreased by 5%.

Presently (2011) the raw wool production is equal to 52.6 thousands of tons on all types of farms. In the last 3 years the wool production decreases in spite of the considerable growth of sheep number (Table 2). The reasons are the following: wool devalued, wool production is unprofitable and demand for wool decreased sharply. Due to these facts in 2000-2011 the wool clips per one sheep

decreased in all types of farms by 0.5 kg (from 3.1 kg to 2.6 kg) and in agro organizations by 1 kg (from 3.2 kg to 2.2 kg).

Mutton production in dead weight in all types of farms was 170,7 thousands of tons in 2011 and it was 51.5 thousands of tons (43.2 %) more than in 2000.

Higher mutton output in dead weight (in 6.5 times) in this period was registered in peasant households.

Some time ago sheep breeding in Russia was mainly (about 80 %) presented by fine-wool sheep targeted for the production of homogeneous wool - thinness 60-64 quality (22-25 microns).

Table 2. Production of main sheep products on various types of farms

Parameter	Year				
	2000	2007	2009	2010	2011
Farms of all types					
Sheep and goats (in dead weight), thousands of tons	140.3	167.9	182.6	184.6	189.0
Wool (in physical weight), tons	40088	52024	54659	53522	52575
Agro organizations					
Sheep and goats (in dead weight), thousands of tons	15.1	13.7	16,5	16.4	17.0
Wool (in physical weight), tons	15144	11040	10847	10541	9529
Households of population					
Sheep and goats (in dead weight), thousands of tons	119.5	136.9	137.1	133.9	134.7
Wool (in physical weight), tons	22755	28948	29398	29113	28833
Peasant households					
Sheep and goats (in dead weight), thousands of tons	5.7	17.3	29.0	34.3	37.3
Wool (in physical weight), tons	2189	12036	14414	13868	14213

Sheep production profitability

Lately the merino wool production thinness 60-64 is unprofitable, 1 kg of wool costs 30-40 rubles (Table 3).

Table 3. Average prices for sheep products realized on farm (average price per year, rubles per a ton)

Year	Sheep		Wool (physical weight)
	live weight	meat 45 % output	
2000	12009	26687	24307
2007	33647	74771	24279
2009	42043	93429	30155
2010	45174	99969	29485
2011	63054	140130	41396
2011/2000, %	525	525	170

To increase the profitability of fine-wool sheep breeding the activities on wool thinning are conducted because merino wool (thinness less than 20 microns) is of greater demand and more valuable. Due to this reason since 2007 on some farms (over 10) of the Stavropol region the fine-wool sheep have been crossbred with fine-wool rams of "doni-merinos" breed brought from Australia. Using of these rams as well as wool thinning ensure the mutton productivity increase from a breed.

The economic weight of mutton has increased in recent years and it is the special feature of the modern world and Russian sheep breeding. Presently in most countries in the world (including Russia) the profit from mutton production is 90% or more and the profit from wool realization is about 10%. So recently more attention is given to the development of early maturing mutton and mutton-wool sheep breeding (mainly crossbreeding) as combining the production of valuable crossbred wool with big amount of high-quality mutton in the best way. In the meat balance of this sector the lamb meat proportion is systematically increasing as the demand for such meat is higher than for meat of older sheep.

Sheep breeding specialization in meat production combining with crossbred wool is the main way of the sector economic efficiency increasing (*Golcblat et al. 1988*, *Erohin et al., 2013).*

Due to this reason in Russia in recent 10-15 years some new mutton breeds using rams of teksel breed, pol-dorset breed, vostochno-frizskaya breed and some other breeds have been bred: tashlinskaya breed, southern mutton breed and western-Siberian mutton breed. Such breeding work continues.

Besides lately the special attention is given to the local hair sheep breeds due to the possibility of mutton production increase. So the sheep of romanovskaya breed, edilbajevskaya breed, karachaevskaya breed and some other hair sheep breeds are of greater demand. The sharp increase in sheep number of these breeds and some other hair sheep breeds testifies this fact. So at 01.01.2001 hair sheep number on farms was 159.2 thousands of heads and in 2011 this number increased to 1118.6 thousands of heads (in 7 times).

The industrial target program "Sheep breeding and goat breeding development for the period of 2012-2014 and up to 2020" (confirmed by the Ministry of Agriculture of the Russian Federation in 2011) declares to increase the sheep and goats number to 28 million of heads, produce mutton - 336 thousands of tons, wool in physical weight - 84 thousands of tons, sheep skins - 8 million skins up to 2020.

There are possibilities to solve these problems. Russia has more than 80 million hectars of pastures and hay fields which can be rationally used mainly by sheep; the available genofond of domestic breeds if used properly may ensure the rapid growth of all types of sheep breeding production; the specialists in production and scientists are able to solve the problems of industry.

The state support will assist producers in agro sector to achieve the goals. The federal budget fund will be 9 569.6 million rubles in total; regional budgets fund – 2 875,4 million rubles. The funds of agro producers for the program realization will be 12 320.1 million rubles.

Conclusions

Based on the analyzes of the situation in Russian sheep breeding, can draw the following conclusions:

Sheep farming is a very important area of livestock in Russia. Number of sheep in all types of farms in was equal to 20 766.8 heads. In the last decade the sheep number in peasant households increased in 8.7 times and in agro organizations decreased by 5%. Presently the raw wool production is equal to 52.6 thousands of tons on all types of farms.

In the last 3 years the wool production decreases in spite of the considerable growth of sheep number. Mutton production in dead weight in all types of farms at 2011, was 43.2 % more than in 2000. Higher mutton output in dead weight (in 6.5 times) in this period was registered in peasant households.

Lately the merino wool production thinness 60-64 is unprofitable.To increase the profitability of fine-wool sheep breeding the activities on wool

thinning are conducted because merino wool (thinness less than 20 microns) is of greater demand and more valuable.

The economic weight of mutton has increased in recent years and it is the special feature of the modern world and Russian sheep breeding. Presently in most countries in the world (including Russia) the profit from mutton production is 90% or more and the profit from wool realization is about 10%.

Sheep breeding specialization in meat production combining with crossbred wool is the main way of the sector economic efficiency increasing.

In recent 10-15 years some new mutton breeds using rams of teksel breed, pol-dorset breed, vostochno-frizskaya breed and some other breeds have been bred: tashlinskaya breed, southern mutton breed and western-Siberian mutton breed. Such breeding work continues.

The state support will assist producers in sheep breeding sector to achieve the goals.

Stanje i perspektive razvoja ovčarstvu u Rusiji

A.I. Erokhin, E.A. Karasev, I.N. Sycheva

Rezime

Jedna od najvažnijih grana poljoprivrede u Rusiji je ovčarstvo. Ovce obezbeđuju sirovine za preradu mesa i tekstilnoj industriji, a proizvodi industrije u ovčarstvu su izvor za narodne zanate. Rase ovaca koje se gaje u Rusiji (39 rasa) imaju različite proizvodne specijalizacije: fina-vune ovaca (14 rasa), polu-fina-vune ovaca (11 rasa), polu-runo ovce (2 rase) i runo ovce (2 rase). Ove rase se gaje u našoj velikoj zemlji, u skladu sa klimatskim zonama. U mnogim ruskim regionima gde uzgoj drugih domaćih životinja je nemoguć, ovce su vodeća vrsta životinja zbog svojih odličnih adaptivnih kvaliteta. Broj ovaca direktno utiče na zapošljavanje lokalnog stanovništva i često je glavni izvor prihoda. U poslednjih nekoliko godina, zbog promene oblika svojine, glavno povećanje broja ovaca je registrovano u privatnim domaćinstvima seljaka. Proizvodnja vune, zbog nerentabilnosti i niske otkupne cene za vunu, dovodi do smanjenja potražnje za vunom i povećanom potražnjom za ovčetinom. Dakle, sada se velika pažnja posvećuje razvoju ranog sazrevanja mesa i meso-vuna pravcu u ovčarstvu. Zato je glavna tendencija razvoja ovčarstva danas uzgoj ovaca kombinujući visoku proizvodnju mesa i vune meleza.

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DOMESTIC PROJECT: CHALLENGES AND STRATEGIES FOR THE SUSTAINABLE DEVELOPMENT OF THE PASTORAL AND RANGELAND SHEEP AND GOAT PRODUCTION SYSTEMS IN IPEIROS (EPIRUS) REGION IN GREECE

Ch. Ligda[1], E. Sossidou[1], I. Tzouramani[2]

[1]Veterinary Research Institute - NAGREF, 57 001 Thessaloniki, Greece
[2]Agricultural Economics and Policy Research Institute -NAGREF, 115 28 Athens, Greece
Corresponding author: chligda@otenet.gr
Invited paper

Abstract: Case studies from four countries of the Mediterranean region (Greece, France, Cyprus and Morocco)are analysed in the frame of the DoMEsTIc project (ARIMNet initiative in EU 7th Framework Programme) in order to assess the associations between the structure of the farming systems and the characteristics of the local sheep and goat breeds with the sector's resilience and sustainability. Three main topics are investigated: i) livestock farming systems, ii) genetic management of the breeds, and iii) economical aspects. The project, still in progress, concluded with some first results regarding the case study in Epirus (GR) that are presented in this paper. The region of Ipeiros (Epirus) is considered as a Less Favoured Area (LFA) where small ruminant production consists one of the major sectors of economy and is mainly based on natural resources.
Data was collected through personal interviews with the farmers. The analysis of data (32 interviewed farmers) demonstrated that the main weaknesses of the sector, are considered the age of farmers and the lack of successors, the lack of commercialization strategies, certification and labelling and the lack of rational organisation of community pastures. In general, the farmers are willing to be engaged in a process that will allow them to sell their products at a higher price. The main strategy that all agree towards the sustainability of the sector, is the need to implement policies to improve the infrastructures in the mountain areas and to take support measures for low input farming systems.

Keywords: Sheep and goats, production systems, sustainability

Introduction

In the Mediterranean region, rangeland and pastoral systems, considered as the traditional way of farming, have been shaped over the centuries both by and for the specific conditions of the environment. In this context, knowledge systems and know-how have developed gradually by interacting to this particular environment *(Bouche, 2011)*. These systems contain elements that can be proved of high value under the new conditions, related with the need for the protection of the environment and biodiversity and with consumer demands on safe and quality products. The necessary evolution of the rangeland and pastoral systems refers to an overall adaptation of their components so that the production activities will provide sufficient income to the farmer in appreciable working and living conditions.All actions should clearly target the recognition of the profession in connection with the position of the farmer in the local communities, the economic, social and cultural sector *(Ligda et al., 2011; Tchakerian, 2007*).

The uniqueness of Mediterranean livestock products, appreciated by a wide range of consumers, and the contribution of the livestock systems to the maintenance of the natural environment as well as of the population in rural areas, are some of the strengths of these systems, which could provide an opportunity for their sustainability and development *(Belibasaki , 2012; Boyazoglu and Morahnt-Fehr, 2001; Casabianca, 2011; Casabianca and Matassino, 2006; da Gama, 2006; Sossidou et al., 2004; de Rancourt et al., 2006).*

The increased economic value will be achieved by higher selling prices but also with the better access to markets, either new ones or by securing access to existing through product differentiation. Mediterranean livestock production systems operate in an environment characterized by a high degree of dynamism and uncertainty. There is a significant rise in market pressure and competitiveness, which threaten the future and survival of the small ruminant sector in the region. Issues related with the loss of farm profitability resulting from an increased cost of inputs and declining meat and milk prices as well as the aging and shrinking farming population factors leading to a reduction of herds and farms, which in turn may worsen with a decrease in subsidies in the EU countries. This situation may also bring about serious social and environmental consequences as sheep and goats contribute to the conservation of harsh marginal areas and to the maintenance of rural livelihoods. On the other hand, in the countries of southern and eastern Mediterranean regions, small ruminant populations are increasing as a strategy to compensate the low productivity of local production systems, raising some environmental concerns because of overgrazing and consequent soil degradation and deforestation. It is therefore necessary to analyse the sustainability of sheep and goat production systems in the region, covering the economic, social and

environmental aspects, including their adaptive capacity to socioeconomic changes. At present, little research is reported in this area. In addition, methodologies of analysis that would enable a thorough understanding of the nature and complexity of the underlying factors are not readily available. Furthermore, climate change is likely to create a number of problems in many areas of animal husbandry (housing, fodder and water availability, disease challenge etc) and threaten the sustainability of many livestock production systems and their associated AnGR *(FAO, 2007).* Mediterranean livestock farming systems have adapted to these and have taken advantage of the diversity of lands that characterise the Mediterranean area, interacting strongly with the land and the environment. However, current knowledge is limited and additional research in this domain is required.

Case studies from four countries of the Mediterranean region (Greece, France, Cyprus and Morocco) are analysed in the frame of the DoMEsTIc project in order to assess the associations between the structure of the farming systems and the characteristics of the local sheep and goat breeds with the sector's resilience,competitiveness and sustainability. Three main topics are investigated: i) livestock farming systems, ii) genetic management of the breeds, and iii) economical aspects. The project is still on going and the data analysis is not completed; the objective of the presentarticle is to analyze the complexity of sheep and goat sector in Epirus and suggest the necessary strategies for the sustainable development of the sector.

Material and Methods

In Greece the field work was carried out in the region of Ipeiros, which is is considered as aLess Favoured Area (LFA) i.e. areas with a high agricultural potential but with limited access to infrastructure and markets, low population density, or other socioeconomic constraints. In the region small ruminant production consists one of the major sectors of economy and is mainly based on the natural resources of the area. In the mountainous and semi-mountainous regions, the sheep and goat production systems are based in grazing, in certain cases with transhumance, mainly in the mountainous zones. The sheep population presents high variation in morphology and production characteristics and shows excellent adaptability to the local environment *(Georgoudis et al., 2006; Hatziminaoglou, 2005; Ligda et al., 2009; Tzouramani et al., 2011; Belibasaki et al., 2012*).

The survey was conducted in collaboration with the Association of Pastoral Farmers of Epirus (EMKH) and focused to the three municipalities of Ioannina County, Ioannites, Metsovo and Pogoni with the highest concentration of sheep and goats farming. 73 farms have their main installation (winter) in the county of

Ioannina, from which 57 are located in the three municipalities mentioned above. From this selected area data were collected from 32 farms, with personal interviews with the farmers.

The questionnaire that was developed for the survey includesseveral sections covering the farmer's profile, the farm and the production system (including information on management, feeding, breeding and reproduction, health management), information on performance recording, the animal products and the economics of the farm. The last section was devoted to the challenges and strategies aiming to prioritize the challenges and threats to the sector and define the strategies that are needed to achieve the sustainability according the farmer's perception. The analysis of the data is still in progress and in this paper we focus on the last section of the questionnaire i.e. the challenges and strategies This section includes a list of parameters (possible threats and strategies) that the interviewed farmer had to rank from 1 to 5, according the importance of the specific parameter. The list has been designed in a way to be used for all four case studies that were included in DoMEsTIc project. In total 12 variables were included as threat parameters, and 7 variables in the strategies section. The list of variables is included in Table 1. The results that are discussed below include the preliminary statistical analysis of this section using SPSS ver.20 (IBM SPSS Statistics 20) and a SWOT analysis (Strengths, Weaknesses, Opportunities, Threats) which is applied as a valuable adjunct to experimental research for it helps to organize the information and analyze the representation of physical processes(*Sossidou et al., 2007*).

Results and Discussion

Preceding the presentation and discussion of the SWOT analysis results, an overview of the current situation of the sector and a brief description of the production systemis provided. This description is based on the data collected during the survey, corresponding to other sections of the questionnaire. Sheep population in the region is consisted mainly with different crosses of local mountain sheep breeds among them, or in some cases with Chios, Lesvos, Karagouniko or Frizarta. There are few flocks with pure bred animals of the local sheep breeds of Katsika (Karamaniko), and Kalarritiko, which are considered endangered due and are included in situ conservation programmes. The main reason of farmers' preferences to the specific breeds or populations is their adaptation in the environment and the production system. A key difference is observed between farmers that raise the Katsika and Kalarritiko breeds, who express their strong belief on their breed's special characteristics, both in terms of phenotype, but also on their quality characteristics. In these cases heritage and

tradition is considered as an important factor for the choice of breeds, but also the incentives they receive for the loss of part of their expected income due to the smaller productvity.The goat population is considered as the local type of goats while there are also few herds with the Skopelos breed, Alpine, or Damascus. In the survey, seven farmers raise only goats, while 5 of them use autochthonous breeds, the local goat and one the Skopelos breed. In the majority of the sheep farms, goats are also raised together with the sheep flock.

The production system is based on grazing, either in the land around the farm, either rangelands or cultivated lands, and the community pastures. The land used for the sheep and goat farming varies throughout the year, as from middle June until the beginning of November, the flocks/herds move to the mountain pastures.

In general the management system is divided in 3 periods :

1. December until February : the animals are housed and complementary feeding is provided, forages and concentrates
2. March until May : the animals move to short distances and housed during the night. Grazing is the main feeding resource.
3. From end of May or June, until November : the animals move to the mountain pastures, more than 1200m. Feeding is exclusively based on grazing. The mountain pastures are mainly community lands and are divided in different parts for each farmer.

Milk production is the main source of income for sheep and goat farmers. The 75% of the milk production is directed to the principal dairy industry of the region DODONI (a cooperative until November 2012), while other small dairies receive smaller quantities. Milk is transformed to feta (PDO), kefalograviera (PDO), galotyri (PDO), other cheeses and yoghurt. Very few farmers produce dairy products on farm for direct selling, however all farmers produce cheese, yogurt and other products for self consumption. Regarding meat production, two main channels are described; the majority of the production is directed to the market through the wholesaler, while the remaining is sold directly to the consumer, or is self consummated. The percentages can vary between the farmers. The prices are unified in the region, formed by few actors.Although farmers have a high appreciation of their products, in most of the cases (only few exceptions) they do not have special agreements which differentiate the prices according to the quality, neither for meat or milk products. In general the farmers are willing to be engaged in a process that will allow them to sell their products at a higher price. There are some initiatives towards this direction, mainly for meat production.

The descriptive statistics analysis of the questionnaire revealed the following parameters as the most frequently reported threats for the sustainability of the sector: Aging of the farmers and lack of successor, the existence of

predators, the rational management of pasture lands and animal health and disease control (most frequent ranking of the threat is 5 in all four parameters). 45.2% of farmers consider as a major threat (rank 5) the aging of the farmers and the lack of successors, and about the same percent the existence of predators. Regarding the threats that are linked to the technical interventions (breeding and reproduction, feeding, health and disease control and management of pasture lands), despite the recognized need that these should be enhanced, only for the two of the variables, management of pasture landsand health and disease control the most common rank is 5, and in total 58% ranked the management of pasture lands as an important threat (4 or 5) and 44% of the farmers ranked the health and disease control as an important threat.

Regarding the strategies to be followed, these can be divided to the general policy measures that are necessary to ensure the sustainability of the sector and the specific interventions. According to this division, the policy measures to support promote extensive farming have been ranked as the most important priorities, with 95.8% of the farmers ranking this strategy as 4 or 5,the need to take measures to support and promote extensive farming, while 82% of the farmers consider as a first priority (5) (92.8% ranked as 4 and 5), the need for measures to improve the infrastructures in the mountainous areas. Certainly, this could mean that it was easier for the farmer to identify as a necessary strategy a general framework that would improve his conditions of working and living, which includes all measures from improvements on the installations in the summer pastures, the connecting roads and the communication and the technical support. The third strategy that was also ranked as an important priority, the improvement of support and advisory services (96% of the farmers ranked this variable as 4 or 5), followed by the development of market oriented policy (58% of the farmers, ranked as 4 or 5 this variable).On the other hand, it is observed that more specific policies regarding the differentiation of production, the support of other activities (as agrotourism) and consumer awareness, which will lead to the increase of value of the products and consequently to the sustainability of the production system, are ranked as medium priorities (mode value=3), mainly because the farmers considered that these measures, are included in the general strategies which currently are missing for the region and can be detailed and specified according to each case, provided that a healthy framework that promotes the extensive farming is being established. The results are presented in Table 1. Further analysis of the data will reveal the relationships between the different threat factors and the parameters of the production system.

Table 1. Descriptive statistics of threats and strategies						
	Number of replies	mean	median	mode	Frequencies of 5	Frequencies of 4
Threats						
Age of farmers / Lack of successors	31	3.9	4	5	45.2	19.4
Limited availability of labor force	31	2.6	3	3	9.7	3.2
Breeding and Reproduction	30	2.4	2	2	20.0	3.3
Developing feeding program	30	2.3	2	2	10.0	6.7
Animal health and disease control	31	3.1	3	5	32.3	12.9
Rational management of pastures	31	3.3	4	5	19.4	38.7
Limited infrastructures f or processing	30	3.0	3	3	20.0	16.7
Limited infrastructures for slaughtering	30	2.9	3	4	3.3	43.3
Low promotion of labeled products	29	3.6	3	3	34.5	13.8
Long distances to markets	30	3.5	2	2	10.0	13.3
Changes in CAP	30	2.6	2	2	16.7	3.3
Predators	27	4.3	4	5	44.4	40.7
Strategies						
Marke toriented policy	24	3.7	4	5	37.5	20.8
Differentiation of production	24	3.7	3	3	33.3	12.5
Consumer awareness	24	3.5	3	3	20.8	25.0
Support extensive farming	24	4.5	5	5	58.3	37.5
Support and advisory services	26	4.6	5	5	50.0	46.2
Improve infrastructures	28	3.6	3	3	82.1	10.7
Other activities	24	3.0	3	3	25.0	20.8

In addition to the descriptive statistics, a SWOT analysis which is presented in Table 2, aimed to organize the different parameters and analyze the current situation of the sector in Ipeiros. The analysis was enriched with information from other stakeholders in the region.

Table 2. SWOT analysis of Ipeiros case study

STRENGHTS ▪ Rich biodiversity and natural environment ▪ Local farm animal breeds adapted to the environment ▪ Low input production systems ▪ Exploitation of pastures ▪ Unique flora that give special aromas to the agricultural products ▪ Highly appreciated by the consumers products (milk and meat)	**WEAKNESSES** ▪ Low population density – ageing of farmers – lack of successors ▪ Lack of appropriate breeding strategies ▪ Lack of rational organisation of community pastures ▪ Lack of organizations of professionals – and of co-operation between the competent authorities ▪ Lack of commercialization strategies - Limited access to markets and infrastructure – Lack of certification and labelling ▪ Lack of social services (education, health) ▪ Luck of integrated policies for the protection of the biodiversity ▪ Lack of vocational training ▪ Low agricultural output prices and market distortions
OPPORTUNITIES ▪ Improve the infrastructures in the mountainous areas ▪ Incorporate innovative methods in the traditional production systems ▪ Development of suitable breeding programmes ▪ Protection of the environment along with the implementation of good farming methods ▪ Implementation of integrated management system, improve farm animals' health and welfare ▪ Development of market oriented policies -Production of certified agricultural products ▪ Diversification of economic activities ▪ Co-operation between the different stakeholders	**THREATS** ▪ Depopulation of the areas and movement of livestock farming from the mountainous to plain zones ▪ Low productivity - Low income ▪ Lack of professional organisations ▪ Economic exclusion ▪ Social exclusion

Conclusion

The main weaknesses of the sector, according to the interviews, are considered the age of farmers and the lack of successors, the damages from the

predators, and the lack of technical support for the rational organisation of community pastures, and health and disease control. Although the lack of promotion strategies for certified and labelled products is not included in the most important threats, the need for such strategies has been identified and in general the farmers are willing to be engaged in such processes. The main strategy that all agree towards the sustainability of the sector, is the need to implement policies to improve the infrastructures in the mountain areas, take support measures for low input farming systems and enhance support and advisory services.

For the effective promotion of sustainable sheep and goat farming in LFAs of Ipeiros, policies can be summarized as following:

- Improving competitiveness of farming by developing infrastructure related to development of agriculture.
- Enhancing the professional organisation of farmers and the collective organisation.
- Encouraging the implementation of integrated strategies for sustainable development.
- Supporting farmers who participate in food quality schemes, animal welfare standards and agri-environment measures.
- Supporting the adaptation and modernization of education, training and employment systems to assist regions taking into account the general needs of areas facing structural difficulties with regard to economic and social conversion.

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DoMEsTIc projekat: izazovi i strategija za održivi razvoj ekstenuzivnih i pašnjačkih proizvodnih sistema u ovčarstvu i kozarstvu u region Epir u Grčkoj

Ch. Ligda, E. Sossidou, I. Tzouramani

Rezime

Studije iz četiri zemlje regiona Mediterana (Grčka, Francuska, Kipar i Maroko) su analizirani u okviru DoMEsTIc projekta (ARIMNet inicijativa, 7. Okvirni program EU) u cilju procene veze između strukture poljoprivrednih sistema i karakteristike lokalnih rasa ovaca i koza sa stanovišta održivosti t sektora. Tri glavne teme su istraživane 1) sistemi u stočarstvu, 2) genetsko upravljanje rasama, i 3) ekonomski aspekti. Projekat je još u toku, a neki preliminarni rezultati u vezi sa studijom u Epiru (GR) su prikazani u ovom radu. Region Ipeiros (Epir) se smatra manje pogodnim područjem - Less Favoured Area (LFA), gde proizvodnja odnosno uzgoj malih preživara čini jedan od glavnih sektora ekonomije i uglavnom se zasniva na prirodnim resursima.

Podaci su prikupljeni putem ličnih razgovora sa poljoprivrednicima. Analiza podataka (32 intervjuisana poljoprivrednika) pokazala je da su glavne slabosti sektora starost poljoprivrednika i nedostatak naslednika, nedostatak strategije komercijalizacije, sertifikacije i obeležavanja proizvoda, i nedostatak racionalne organizacije zajednice pašnjaka. U principu, poljoprivrednici su spremni da se angažuju u procesu koji će im omogućiti da prodaju svoje proizvode po višoj ceni. Glavna strategija oko koje se svi slažu da će omogućiti stabilnost ovog sektora, jeste potreba da se sprovede politika za poboljšanje infrastrukture u planinskim oblastima i preduzmu mere podrške malim poljoprivrednim sistemima.

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COMPARISON OF THE EXTERIOR CHARACTERISTICS OF THE ENDANGERED SHEEP BREEDS IN MONTENEGRO AND REPUBLIC OF CROATIA

B. Marković[1], Z. Antunović[2], J. Novoselec[2], M. Marković[1], Ž. Klir[2]

[1]Biotechnical Faculty, University of Montenegro, St. Mihaila Lalića 1, 81000 Podgorica, Montenegro
[2]Faculty of Agriculture in Osijek, University of J.J. Strosmayer, St. P. Svačića 1 d, 31000 Osijek, Croatia
Corresponding author: B. Marković, e-mail: bmarkovic@t-com.me
Invited paper

Abstract: The aim of the study was to determine the exterior characteristics of the endangered autochthonous sheep breeds in Montenegro and Croatia. The study was conducted in Montenegro on 38 heads of Zeta zuja breed and in Croatia on 40 heads of Dubrovnik sheep breed. Both breeds are reared in the areas of Mediterranean climate condition, Zeta zuja near Podgorica (Zeta-Bjelopavlici plain), while Dubrovnik sheep in the Dubrovnik-Neretva County. Body measures of sheep (height of withers – HW, chest circumference – CC, body length – BL, chest width – CW, chest depth – CD, height of rump – HR, pelvis width – PW and bone circumference – BC) were determined by the Lydthin's stick or stock tape, and body weight by balance. Also, indices of body development in both breeds were calculated. The results of height of withers (63.10 cm and 63.15 cm), height of rump (65.04 cm and 65.37 cm) were similar in both breeds ($P>0.05$), while body weight of Zeta žuja (37.10 kg) and Dubrovnik sheep (46.50 kg), body length (59.41 and 65.0 8cm) and pelvis width (15.0 and 18.3 cm) were significantly different ($P<0.01$). Value of body format index of Zeta zuja (94%) indicates its high format, while for Dubrovnik sheep (103%) square to long format. According to the results of body development investigated breeds are small to medium developed breeds. As indigenous and endangered, these breeds have to be preserved and by better nutrition and selection their production performances have to be improved.

Key words: sheep, endangered breeds, exterior characteristics

Introduction

Sheep production is a very important segment of livestock production, particularly in countries or regions where pastures and natural meadows dominate. Areas that cannot be used for intensive agricultural and crop production could be efficiently exploited by sheep *(Mitic, 1987)*. Economic importance of sheep breeding is based on their biological characteristics that enable them to transform relatively sparse vegetation of various pastures successfully into high-quality products: meat, milk and wool.

Sheep production in Montenegro is also important sector of livestock production, especially because less productive areas (natural meadows and pastures) prevail in total agricultural land (88%). The production is based on the about 200.000 breeding heads, mostly distributed in the central and northern-mountainous part of Montenegro. Sheep production relies on rearing of the local Pramenka breeds: Pivska or Jezeropivska and Sjenicka breed, as the dominant, while Bardoka, Ljaba, Sora and Zeta zuja have much smaller share in total population *(Markovic et al. 2009)*. All of these breeds are very well adapted to the local climate and geographic conditions of rearing and they are very valuable from the preservation of genome of autochthonous breeds point of view. Rearing area of Zeta zuja breed is very limited, only flat area around Podgorica (Zeta–Bjelopavlici plain and Basin of Skadar Lake). Population of Zeta zuja has rapidly been decreased, thus today there are only few tens of purebred heads. It is the most endangered breed of sheep in Montenegro with high risk of extinction. This breed has a very good resistance and excellent adaptability to the very hot climate in the rearing area. Zeta zuja, as autochthonous sheep breed in risk of extinction, is included in the National program of *in situ* conservation of animal genetic resources. Program of *in situ* conservation has been started in 2009 and so far it includes three flocks with total 95 heads of this breed.

In the Republic of Croatia, the Central Register of sheep and goats in the end of year 2012 included 637280 breeding sheep. In Croatia 16 breeds of sheep are reared, including 9 indigenous and 7 foreign breeds. The majority of the sheep population is based on indigenous breeds with the main aim of meat production, especially lamb meat. From indigenous sheep breeds only Dubrovnik sheep have the character of an endangered breed. It was developed by random crossing of domestic sheep Pramenka with imported Spanish, French and Italian Merinos with aim to improve quality and quantity of wool. Dubrovnik sheep is the most endangered Croatian indigenous breed. In recent years, *in situ* conservation of Dubrovnik sheep breed has been conducted. In Croatia, according to the report of *Croatian Agricultural Agency* (2013), 702 heads of Dubrovnik breed are reared, that is only 1.56% of the total number of sheep. Area of breeding is in Dubrovnik -

Neretva County, in 34 registered breeders' flocks, with an average flock size of 20 breeding animals.

Considering the endangerment of Dubrovnik sheep in Croatia and Zeta zuja breed in Montenegro, key question is how to provide long term conservation and sustainable use of them, as well as possibilities to their spreading, not only in traditional areas where they are reared for centuries, but in similar areas of orographic and climatic conditions. Very few papers about Dubrovnik sheep breeding (*Mioč et al., 2003; Antunović et al., 2011a*) and Zeta zuja *(Adzic and Ljumovic 1987 and Adzic and Markovic 2003)* have been published so far.

The aim of this paper is to determine the main body measures and make comparative analysis of exterior characteristics of the existing population of Zeta zuja and Dubrovnik sheep, as well as to compare them with other sheep breeds in Montenegro and Croatia.

Material and methods

The study was conducted in Montenegro on 38 heads of Zeta zuja and in Croatia on 40 heads of Dubrovnik sheep breed. The animals were, 3 to 6 years old. The study was conducted on family farms near Podgorica (Zeta-Bjelopavlici plain) in Montenegro and in the Dubrovnik-Neretva County in Croatia. The sheep were healthy and in satisfactory condition. System of rearing was extensive or semi extensive, based on maximal use of natural pastures which are in summer months due to arid climate condition sometimes very poor. Dubrovnik sheep after returning from pasture to the barn used to receive 150 g of corn per animal.

The research was conducted at the end of summer or beginning of autumn. Measuring and estimation of the exterior characteristics of the sheep were conducted in morning hours before animals go out to pasture. Body measures of sheep (height of withers - HW, chest circumference - CC, body length-BL, chest width - CW, chest depth - CD, height of rump - HR and bone circumference - BC, PW - pelvis width, pelvis height - PH) were determined by the Lydthin's stick or stock tape, while the body weight was measured by balance. Also, the following indexes of body development in both breeds were determined: IBF - index of body format (HW/BL*100); IC – index of chest (CW/CD*100); ID - index of depth (CD/HW*100); IM - index of massiveness (CC/HW*100), IBP – index of body proportion (BW/HW*100). Results were analyzed by using SAS (9.3) MEANS procedure.

Results and discussion

The exterior of Zeta zuja breed is very characteristic and easy to determine: yellow – brown colored hair cover face, ears and lower parts of legs. It is named "yellow face``, while the wool is white colored. The rams have strong triangular horns, while the ewes are usually hornless. Dubrovnik sheep are in the most cases (90%) totally white colored (wool and hair on head and legs) and in some cases animals could be black colored (5-10%). Head is moderately long with visible convex profile of frontal and nasal bones.

The results of body measures, presented in table 1, show that the autochthonous breeds belong to small-sized (Zeta zuja) or medium-sized breeds (Dubrovnik sheep), with average body weight of 37.1 kg and 46.6 kg, respectively, with significant differences (P<0.01) between these two breeds.

Table 1. Body measures of Zeta zuja breed (n=38) and Dubrovnik sheep (n=40)

Body measures	Zeta zuja			Dubrovnik sheep			Differ. of means
	Mean	SD	SE	Mean	SD	SE	
Body weight (BW), kg	37.10	5.05	0.82	46.57	8.22	1.30	**
Height to withers (HW), cm	63.10	2.80	0.45	63.15	3.38	0.53	ns
Height to rump (HR), cm	65.04	2.65	0.43	65.37	2.72	0.37	ns
Chest circumference, (CC) cm	81.85	4.45	0.72	84.16	5.58	0.88	**
Body length (BL), cm	59.41	2.86	0.46	65.08	2.56	0.39	**
Chest width (CW), cm	15.13	1.35	0.22	19.59	1.96	0.31	**
Chest depth (CD), cm	27.26	1.63	0.26	30.14	2.03	0.32	**
Pelvis width (PW), cm	15.00	3.14	0.51	18.63	1.48	0.23	**
Bone circumference (BC), cm	7.60	0.44	0.07	7.23	0.24	0.04	*

SD- standard deviation; SE- standard error, ** significant differences (P<0.01), *(P<0.05), ns – non significant differences (P>0.05).

The height to withers (63.10 cm and 63.15 cm) and height to rump (65.04 cm and 65.37 cm), without significant differences between breeds (P>0.05), indicate quite similar potential for growth and body format in these breeds. The measures: length of body, chest circumferences as well as all measures that significantly determines body weight of animals (chest width, pelvis width and chest depth) were significantly higher in Dubrovnik sheep than in Zeta zuja. These measures indicate better growing performances of Dubrovnik sheep, what is probably result of its crossing with some merino breeds during process of creation

of this breed, as well as better condition of rearing, particularly feeding. From the other side, smaller values of chest and pelvis width and chest depth of Zeta zuja breed indicate that it is typical representative of the primitive coarse wool breed. Zeta zuja breed, as small and closed population, is exposed to inbreeding and it has insufficient feeding as well.

Comparison of these results with the results from the previous research for Dubrovnik sheep and Zeta zuja, (Table 2) shows higher values of body weight and most exterior characteristics in this latest research. Constant improvement in conditions of rearing and feeding in last period is, most probably, the main reason for these differences. Only the results of the chest width of Zeta zuja are significantly smaller in this research than in the previous once, which is most probably a consequence of inbreeding in small population. Comparison of the results of Dubrovnik sheep with other Croatian sheep breeds, such as Tsigai (*Antunović et al., 2011b*), Lika Pramenka, Istrian Pramenka, Dalmatian Pramenka, Cres, Krk and Pag sheep (*Mioč et al., 2007*) shows that Dubrovnik sheep can be put into medium developed Croatian breeds of sheep.

Table 2. Body measures of Dubrovnik sheep and Zeta zuja in the previous researches

Data sources	WH, cm	BL, cm	CW, cm	CD, cm	CC, cm	BC, cm	BW, kg
	Dubrovnik sheep						
Rako, (1949)	59.05	61.48	17.48	27.18	80.15	7.33	32.94
Mioč et al. (2003)	60.12	65.05	19.81	30.32	86.45	7.54	47.93
Antunović et al. (2011a)	62.58	65.07	19.42	30.15	83.98	7.21	45.80
	Zeta zuja						
Adzic and Ljumovic (1987)	57.8	58.8	18.17	26.6	77.6	7.0	37.1
Adzic and Markovic (2003)	54.14	55.7	16.24	24.48	70.05	7.43	31.1

The results of body development of Zeta zuja breed in comparison with other indigenous breeds of Pramenka group on the territory of Montenegro *(Adzic and Ljumovic 1987, Ljumovic 1956, Markovic et al 2011)*, as well as Pramenka breeds in the Region (*Mioc et al. 1997)*, indicate that this is one of the smallest breed. Only the results of body measures of Karakacan breed are similar to Zeta zuja *(Mitic 1987)*.

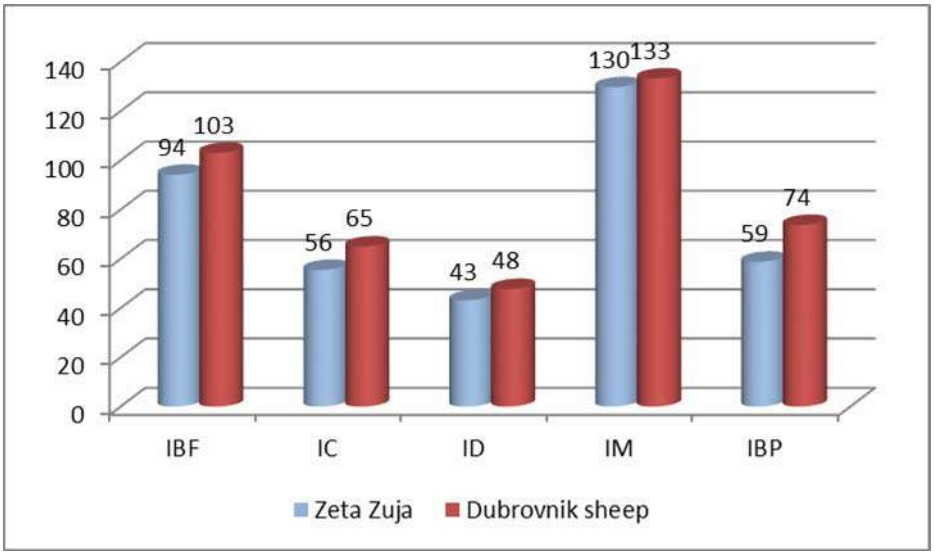

Figure 1. Indices of body development of Zetska žuja and Dubrovnik sheep
(IBF - index of body format, IC – index of chest; ID- index of depth, IM-index of massiveness, IBP – index of body proportion)

Body indexes additionally proof body development and conformation of investigated indigenous sheep breeds (Figure 1). Calculated values of IBF show that Zeta zuja has high shape, while Dubrovnik sheep long shape. Indexes of chest, depth and massiveness indicate that Dubrovnik sheep has a little bit better growth performances, while BPI index indicate that both breeds had satisfactory physical condition. Inclusion of some concentrate feed in the diet would be necessary in order to improve productivity, especially in drought period of the year.

Conclusion

On the basis of the results for the exterior characteristics and their comparison with other breeds in both countries, Dubrovnik sheep is medium-developed breed, while Zeta zuja small-sized breed. Both breeds are very specific indigenous breeds, even endangered due to small effective population size, especially Zeta zuja. Thus, both breeds have to be under control and included into the programs of *in situ* and *ex situ* conservation. In order to improve body performances and production traits, inclusion of some concentrate feed in the ratio, as well as careful selection would be necessary.

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Uporedna analiza eksterijernih karakteristika ugroženih autohtonih rasa ovaca u Crnoj Gori i Republici Hrvatskoj

B. Marković, Z. Antunović, J. Novoselec, M. Marković, Ž. Klir

Rezime

Cilj ovih proučavanja bio je da se determinišu eksterijerne karakteristike ugroženih rasa ovaca u Crnoj Gori i Republici Hrvatskoj. Istraživanja su provedena na 38 grla zetske žuje u Crnoj Gori i 40 grla dubrovačke ovce u Hrvatskoj. Obe ove rase se gaje u uslovima mediteranske klime, zetska žuja u okolini Podgorice (Zetsko-bjelopavlićka ravnica), dok dubrovačka ovca na području Dubrovačko-neretvanskog kantona. Uzimanje telesnih mjera i mase tela vršeno je ujutro, pre izlaska ovaca na pašu. Telesne mere (visina grebena, visina krsta, obim grdi, širina grudi, dubina grudi, širina karlice i obim cevanice) merene su lidtinovim štapom ili pantljikom, dok je masa tela merena vagom. Utvrđeni su i indeksi telesnih mjera za obe proučavane rase. Vrednosti utvrđene za visinu grebena (63,10 cm i 63,15 cm) i visinu krsta (65,04 cm i 65,37 cm) su bile slične u obe rase i nije bilo značajnih razika ($P>0,05$), dok su masa tela (37,10 kg i 46,50 kg), zatim dužina trupa (59,41 cm i 65,08 cm) i širina karlice (15,0 cm i 18,3 cm) bile značajno manje u zetske žuje nego u dubrovačke ovce ($P<0,01$). Indeks formata tela ukazuje da zetska žuja (94%) ima visoki format, dok dubrovačka ovca (103%) kvadratičan do dug format. Rezultati telesne razvijenosti proučavanih rasa ukazuju da su zetska žuja i dubrovačka ovca male do srednje razvijene rase, ali da ih kao autohtone treba očuvati, a boljom ishranom i selekcijom unaprediti njihove performanse.

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OPTIMIZATION OF ENERGY AND PROTEIN LEVEL IN DIETS FOR FATTENING LAMBS

D. Ružić-Muslić[1], M. P. Petrović[1], M. M. Petrović[1], Z. Bijelić[1], V. Caro Petrović[1], V. Pantelić[1], P. Perišić[2]

[1]Institute for Animal Husbandry, Belgrade-Zemun, 11080, Serbia
[2] Faculty of Agriculture, University of Belgrade, Zemun, Serbia
Corresponding author: muslic.ruzic@.gmail.com
Invited paper

Abstract: Sheep have the ability to transform the relatively low quality food into high quality protein of animal origin (meat, milk). To increase the efficiency of feed utilization and minimize pollution of the environment, it is necessary to better understand the needs of lambs in nutrients (primarily energy and protein) and their interaction in the organism. Optimal ratio between energy and protein in the diet of lambs optimizes microbial protein synthesis and maximum retention of degradable nitrogen in the rumen. The excess of the energy consumed in the diet, will be transformed into fat, as the degree of increase of muscles and bones is limited. Feeding lambs diets deficient in the protein, leads to the formation of large amounts of fat in the body. The decline in the efficiency of energy use, in case of the surplus of protein in the diet, is associated with energy consumption for the formation and excretion of urea. The results of our study showed that lambs crosses of Pirot Pramenka Wuerttemberg x Ile de France of average body weight of about 15 kg and 30 days of age and fed with a diet containing 4% beef tallow (7.71 MJ NEM and 16% CP) realized significantly ($P<0.05$) higher average daily gain and final body weight compared to animals that have consumed feed with 6% beef tallow and 7.94 MJ NEM. Also, animals on this treatment realized the best conversion of dry matter, protein and energy. The above nutritive treatment did not significantly affect the meat yield, the yield of meat per categories and morphological composition of the carcass side.Bearing this in mind, the aim of this paper is to present our own, and the results of other authors, related to the optimization of energy and protein levels in diets for fattening lambs.

Keywords: lambs, protein, energy, growth, yield, meat quality

Introduction

Interest in increasing the efficiency of lamb meat production, on the one hand, and the concern about the quality of meat, on the other hand, have motivated significant research efforts in the direction of modeling the composition of lambs through diet. This issue has important implications in several fields, including energy metabolism and protein and their interdependencies with the aim to develop a diet that optimizes the relationship between energy and protein in diets for fattening lambs (*Pittroff et al., 2006*).

Energy, quantitatively, is the biggest factor limiting livestock production. Meeting the needs of lambs in energy is the largest item in the cost of food. Hence, the efficiency of use of energy, in terms of quantity and cost of production, is a very important question. Animals can be supplied energy through partial or complete oxidation of carbohydrates, fats and proteins consumed and absorbed from food.

Most of the soluble (sugars and starches) and insoluble carbohydrates (cellulose and hemicellulose) are fermented, through the action of microbial enzymes, into volatile fatty acids (acetic, propionic and butyric), which are absorbed through the rumen wall into the blood, and transported to the different body tissues (*Jovanović et al., 2001*). Catabolic processes (supplying the energy to the body) and biosynthetic processes (biosynthesis of fat from acetic and butyric acids or the synthesis of glucose from propionic acid) take place in them.

As a second source of energy in diets for fattening lambs, fat is used. Because of the small volume of the digestive tract of lambs on one side and a large increase in the intensity of growth on the other, in order to ensure an optimal level of energy in diets for fattening lambs, it is resorted to using some non-conventional feedstuffs such as fats of animal and vegetable origin (tallow, lard, soybean oil) (*Ruzic, 1997*). Researchers in the UK (NRA) have shown that with 4-6% added fat in the meal ruminants produce 34-45% less methane in the rumen, which results in a savings of 2-5% in energy and the big savings in the cost of fattening.

The role of energy involves the provision of requirements for maintenance (blood circulation, respiration, activity of the nervous system, maintenance of muscle tone, thermoregulation, performance of active absorption and transport of chemical compounds, replacement of damaged tissues, the production of hormones and vitamins) and production functions in lambs. While the requirements for maintenance represent a function of the body weight of lambs, increase and ratio of protein and fat in formed gain are main determinants of the energy requirements for production *(Jovanović et al., 2001)*.

On the other hand, the protein is also a critical nutrient for young animals growing and most expensive food component. Proteins play a fundamental role in

the body of lambs. They participate in the formation and maintenance of tissues, muscle contraction, transport of nutrients and synthesis of hormones and enzymes (*Santos et al., 2006*).

Longer protein deficiency results in a decrease in the efficiency of feed utilization and low immunity of animals (*Santos et al., 2006*). Excess protein can cause a loss of energy. This means that the availability of energy is regulated by adequate protein in the diet. The energy deficit delays puberty, reduces growth, fertility, weight and other production parameters of animals (*Resende et al., 2006*).

Optimal balance between energy and protein in the diet of lambs optimizes microbial protein synthesis and maximum retention in the rumen of degradable nitrogen (*Sinclair et al., 1993*). When the rate of ammonia formed exceeds the rate of fermentation of carbohydrates, nitrogen is not used effectively and a high percentage of nitrogen is excreted through urea (*Huber and Herrera-Saldana, 1994*). Contrary, when the rate of fermentation of carbohydrates exceeds what is metabolised in the rumen and liver, results in acidosis (*Yokoyama and Jonson, 1988*). The effects of protein-energy relationare reflected in the energy balance, and the nature of weight gain (*Pittroff et al., 2006*).

Effect of the level of energy and protein in the diet on performance of fattening lambs

Finding the optimal level of energy and protein in diets for fattening lambs, in order to maximize production in sheep, is the subject of numerous studies in the country and abroad.

In order to achieve higher and more cost-effective growth of lambs, the question of the level of energy is the simplest regulated by adding a by-product of the slaughter industry (animal fat, beef fat) in feed mixtures for feeding offspring. Until recently, in order to adequately supply of sufficient energy, the use of large amounts of carbohydrate nutrients was recommended in ruminant nutrition, that are introduced into the concentrate mixtures. However, this diet had a negative impact on the course of fermentation in the rumen, lowering the pH, which can lead to the incidence of bloat and acidosis. In contrast, the fat in the diet changes the surface tension/voltage of the fluid in the rumen and reduces the tendency of foaming and incidence of bloat. Further comparison of addition of carbohydrate and fat as energy sources, points out the advantage of fat considering that during the fermentation of carbohydrates up to 14% of the energy available in food can be lost, through methane that is unusable for animals (*Ružić, 1997*). The studies (*Ružić, 1997; Ružić et al.1999*) have confirmed that the lambs that consumed isoprotein forage mixture (16% TP) with 4% beef tallow and 7.71 MJ NEM achieved significantly ($P < 0.05$) higher average daily gain and final body weight

compared to animals that have consumed feed with 6% beef tallow and 7.94 MJ NEM. Also, animals on this treatment have realized the most favourable feed conversion of dry matter, protein and energy, as shown in Table 1. Rezults similar to ours, in regard to the use of fat as an energy source in diets for lambs, have been obtained by *Ponnampalam et al., (2005)*. They found that the fat content of more than 5% in the mixture had negative effect on weight gain of lambs as a result of depressed activity of fatty acids on the rumen microflora and fiber digestion.

Table 1. Production performance of fattening lambs (*Ružić, 1997*)

Traits	Energy levels, MJ NEM		
	7.29	7.71	7.94
Body weight at the beginning of the trial, kg	14.97±2.64	15.12±2.58	15.15±2.82
Age at the beginning of the trial, days	30	30	30
Body weight at the end of the trial, kg	29.81±4.07	31.19^{a}±3.36	29.86^{b}±5.66
Total gain, kg	14.84±1.31	16.07^{a}±1.52	14.71^{b}±1.34
Average daily gain, kg	0.280±1.1	0.303^{a}±1.21	0.277^{b}±1.10
Consumption of dry matter kg/kg of gain	2.68	2.58	2.93
Consumption of total proteins kg/kg of gain	481	450	514
Consumption of NEM, MJ/kg of gain	19.07	18.36	20.71

The difference between a and b is statistically significant at the level ($P<0.05$)

From the perspective of the impact of different energy levels on performance of fattening lambs, *Haddad and Hussain (2004)* examined the effects of two energy levels: 2.92 and 2.40 in Mcal/kgday in isoprotein diet on performance of Awasi lambs, of average body weight of 16.7 kg, determined the final weights: 33.4 : 27.9 kg, and the average daily gain: 0.258 : 0.178 kg, respectively. Feed conversion rate was 3.8 : 5.4 kg/kg gain. Therefore, diets with higher energy level had an impact in improving growth and efficient feed utilization. Similar results are obtained by *Saikia et al. (1995), Shahjalal et al. (2000)* who conducted tests on goats. *Hassan et al. (2011)* carried out the experiment on 18 lambs, aged 3 months, with an average body weight of 22.0 kg, fed isoprotein rations (16.5% TP), with different levels of energy: 10.5 MJ ME/kg/DM : 8.5 MJ ME/kg/DM. The lambs on these treatments realized average daily gain (ADG): 0.282 : 0.193 kg, while the DM conversion was: 5.5 : 9.0 kg/kg gain. *Sayed (2011)* examined the impact of different energy levels (2.90 : 3:20 : 3:50 Mcal/kg) in isoprotein diets (14.7% TP) on the performance of lambs. The aforementioned treatments have achieved average daily gain values: 0.180 : 0.284 : 0.215 kg and feed conversion was: 8.13 : 4.59 : 6.26 kg/kg of gain, respectively.

Haddad and Ata (2009) followed the effects of different levels of energy (2.81 : 2.90 : 2.96 : 3.04 Mcal/kg DM) in isoenergy diets (16% TP) on growth performance of Awasi lambs of average body weight of 17.9 kg. The following average daily gains were realized: 0.244 : 0.250 : 0.185 : 0.161 kg with food consumption: 4.9 : 4.7 : 6.1 : 5.7 kg/kg of gain, resepctively. It is evident that the lambs achieved the best results in the diet containing 2.90 Mcal/ kg DM and 16% TP. So, increasing the energy density of the ration for lambs resulted in improved growth performance and better feed conversion. The explanation for this phenomenon lies in the fact that the increase of energy level enables production of large quantities of ME required for microbial growth, resulting in increased microbial protein synthesis and synthesis of protein in general (*Sayed, 2011*). However, feeding diets containing a high proportion of concentrate feed in ruminant nutrition causes acidosis (*Owens et al., 1998*), which sets new requirements in terms of relations between carbohydrate components as well as protein and energy in diets for feeding fattening lambs. In other words, a balanced diet in terms of protein and energy optimizes growth of lambs and kids. *Dutta et al. (2009)* have studied the effect of different energy : protein relations in the diet on performance of Barbari kids in India. Diets contained 1.98 Mcal/kg DM and 2.16 Mcal/kg DM, and 12% and 14% TP. On treatment with 12% protein, and 2.16 Mcal/kg DM an average daily gain was achieved by 7.2% higher relative to the treatment which included 12% protein and 1.98 Mcal/kg DM, while animals fed diets with 14% TP and 2.16 Mcal/kg/DM achieved growth by 14.5% greater than animals in the control group. In the investigation by *Papi et al., (2011)*, the effects of energy-protein ratio in the diet of male lambs on fattening performance were studied. ME content of treatments was 9.12 : 9.96 : 10.67: 11.34 MJ/kg/DM and protein: 143 : 152 : 161 : 174 g/kg. Lambs, in these treatments, realized the following daily gains: 0.244 : 0.269 : 0.278 : 0.238 kg. Thus, the energy level of 10.67 MJ/kg/DM and protein content of 161 g in diet resulted in the highest daily gain. Poor response of animals to the highest level of energy and protein is explained by incidence of the rumen acidosis. *Karlsson and Martinsson (2011)* indicate that a linear relationship between average daily gain and metabolic energy is stronger than those of the average daily gain and total protein content.

In order to maximize utilization of the genetic potential of high yielding breeds of sheep for meat, in addition to optimal energy: protein ratio in the diet, level of protein non-degradable at the rumen level (NP) is very important. Nutrients whose proteins on a large scale pass through the reticulum-rumen and reach the duodenum non-degraded, cause greater weight gain in lambs, in the presence of sufficient energy (*Zeremski, 1989*). This was confirmed in studies (*Ružić-Muslić 2006, Ružić-Muslić et al., 2007-a, 2007-c, 2007-d*) where the level of non-degradable protein (43 : 51:58%) in mixtures, had significant ($P < 0.01$)

influence on the final success of fattening expressed through average daily gain and final body weight of fattening lambs. The highest daily gain (0.227 kg) and the best conversion of dry matter (3.30 kg) were recorded in lambs on treatment with 58% of NP in the mixture. Similar results, in terms of the effects of non-degradable protein sources, have been achieved by *Orskov et al*., *(1971)*, *Miller (1978), Grubić et al*., *(1991), Walz et al*., *(1998), Peter et al*., *(2000)* and *Memiši et al., (2002*). *Grubić et al., (1991)* who analyzed the relationship between average daily gain and protein values expressed in terms of the total, digestible and non-degradable protein in diet, and found the highest correlation coefficient (r = 0.76) between daily gain and the share of non-degradable protein in the diet, slightly lower between gain and crude protein (r = 0.72) and the lowest between gain and digestible protein (r = 0.68). Similar studies have been conducted by *Haddad et al., (2005)* on 30 Awasi lambs, average weight 17.2 kg, fed isoenergy (2.32 Mcal) and isoprotein diets (16% TP), which contained a different proportion of non-degradable 16.1 (I), 22.9 (ii) and 29.9% (III) of the total protein. It was established that lambs on treatment II achieved an average daily gain of 0.265 kg, while the value of the parameter examined in the treatments I and III was: 0.219 and 0.263 kg. It was concluded that the NP 50.3 g/day is needed for maximum growth of Awasi lambs average body weight 17.2 kg. *Galbraith et al. (1997)* have noticed a difference in the rate of weight loss between the two groups of lambs fed isoenergy diets, which differed in terms of supplementation of fish meal as a source of non-degradable protein. Namely, lambs fed diets without the addition of fishmeal have lost 13.3% of body weight in 48 days, while the lambs that were fed fish meal, lost 10.3%.

The effect of the level of energy and protein in the diet on yield and meat quality of lambs

Increased energy levels (7.29 : 7.71 : 7.94 MJ NEM) in isoenergy mixtures (16% tP) for lambs, did not significantly affect the meat yield, the yield of meat per categories and morphological composition of carcass (*(Ružić, 1997; Ružić-Muslić et al. 2009*). The results of these tests are presented in Table 2. The results are consistent with results of *Jordanoski (1981)* who found that the energy level decreased and increased by 8% in treatments A and C with respect to B, had no significant effect on the meat yield of crosses of Ovčepolje x Merino breeds, on treatments A: B: C: 47.45 : 48.21 : 48.92%, as well as the proportion of muscle in the carcass: 60.09 : 60.45 : 59.32%. The influence of the energy levels in the diet of intensively fattened lambs of Tsigai breed was studied by *Negovanović et al., (1983)*. After slaughter, at the average body weight of 31.0 kg, values were determined for yield of 56.36% and 58.61%, which was not affected by the tested treatments. Different concentrations of energy, achieved through different share of

concentrate mixture: 50, 70, and 90% in diets for Barbari kids, did not affect the yield and carcass traits (*Ryan et al., 2007*).

Table 2. Meat yield and the proportion of individual tissues,% (*Ružić, 1997*)

Indicators	Energy levels, MJ NEM		
	7.29	7.71	7.94
Warm carcass with offal, kg	57.89 ±2.17	58.63 ±3.20	58.46 ±4.53
Meat yield per categories			
Meat I category,%	40.66 ±1.49	40.35 ±1.60	40.01 ±1.36
Meat II category,%	34.76 ±1.45	36.13 ±1.08	35.48 ±0.75
Meat III category,%	24.18 ±1.41	23.25 ±1.55	24.37±1.74
Share of individual tissues,%			
Muscle tissue	52.05 ±4.11	52.28 ±5.38	53.54 ±3.07
Fat tissue	25.06 ±5.76	27.71 ±4.95	26.80 ±2.75
Bone tissue	21.84 ±0.22	19.04 ±3.99	18.59 ±3.09
Connective tissue	0.640 ±0.26	0.66 ±0.19	0.59 ±0.06

However, in studies by *Abdullah and Musallam (2007)* it is established that goats fed high-energy diets have a higher proportion of body fat, as in the present research. *Papi et al. (2011)* suggest that different levels of energy (9:12 : 9.96 : 10.67 : 11.34 MJ DE/kg DM and protein (143 : 152 : 161 : 174 g/kg) in diets for lambs, had no statistically significant effect on the meat yield: 51.4 : 56.3 : 56.4 : 55.8%, while total fat and subcutaneous adipose tissue were the lowest in the first energy level and the highest in the third level of energy and protein. Rezults of research by *Shadnoush et al. (2004)* showed that reducing energy levels by 10% in mixture for lambs, had no statistically significant effect on carcass characteristics. Similar observations are stated by *Shiran (1995)* in Lori Baktiar lambs fed diets with different energy content: 2.1 : 2.3 : 2.5 : 2.7 Mcal. Share of muscle, fat and bone tissue was not under a significant influence of treatment.

In addition to the energy level, the question of the impact of protein levels in diets for fattening lambs, on the yield and quality of meat, was the subject of research of a significant number of researchers in the country and abroad.

Ljumović (1967), examining the impact of protein content in the supplemental concentrate feed mixtures on fattening performances of crossbred lambs Pivka x Württemberg, concluded that the increase in protein levels of 10 to 18% did not have a statistically justifiable influence ($P > 0.05$) on the yield: 40.8 and 41.3. *Šokarovski et al. (1988)* found that the proportion of the total protein of 16 and 18% in the mixture, did not affect the yield, given that the values of the

analogue treatments were: 46.70 and 47.14%. Similar observations were presented by *Haddad et al. (2011)*. In the examination of the impact of the optimal level of total protein: 10, 12, 14, 16 and 18% on growth of Awasi lambs, average body weight of 23.0 kg, the authors have determined the optimal protein content of 16%, and that any increase above this level will not result in improving performance.

Besides the impact of the total protein, the research was conducted related to the impact of non-degradable protein in the diet on yield and meat quality of lambs. *Mekić et al. (1999)* investigated the effect of non-degradable protein (41 : 50 : 60%) in the total mass of diet protein on fattening performance and slaughter results of lamb genotype Ile de France, fattened to the age of 88 days. Average meat yield of warm carcass with head and offal was: 54.16 : 56.54 : 57.36%. Share of I, II and III meat category was: 42.09 : 35.61 : 22.30% in lambs in treatment I and 42.63 : 34.84 : 55.53% for lambs in treatment II and 42.01 : 36.20 : 21.79% for lambs in group III. The level of non-degradable protein in the diet, had no significant effect on the dressing percentage value, meat yield and morphological composition of the carcass side, which has been confirmed in studies *Ružić-Muslić (2006)*. The lambs weaned at 60 days of age and an average body weight of about 18.0 kg were fed isoprotein diets (14% TP), but with different proportions of non-degradable protein: 43 : 51 : 58%, to weight of approximately 35.0 kg. Values of warm carcass yield with offal were: 58.70 : 58.02 : 57.42%, respectively. Share of meat of categories I, II and III was 37.27 : 33.19 : 27.78% in treatment I, 37.35 : 32.67 : 29.59% in treatment II and 37.51 : 32.83 : 29.10% in treatment III. The relative share of muscle tissue compared to the weight of three rib cut, was: 43.52 : 42.27 : 41.92%. Fat tissue was present in following values: 26.68 : 31.76 : 30.68%, and bone tissue: 28.23 : 25.06 : 25.93%. The ratio of meat (total muscle and fat tissue) to bone was: 2.5:1, 2.9:1, 2.8:1. The results obtained related to the characteristics of the carcass and the share of individual tissues showed that they were influenced by the treatment, which is consistent with the results of *Atti and Ben Salem (2008)*. The explanation lies in the fact that the lambs had similar body weight of empty carcass and carcass composition, as they are slaughtered at similar final weights. These parameters depend mainly on body weight at slaughter (*Colomer-Rocher and Espejo, 1972; Atti and Ben Salem., 2008*).

The results of the study of the surface, chemical and technological characteristics of *Musculus longissimus dorsi* (MLD) are presented in Table 3

Energy levels in isoenergy rations for fattening lambs did not affect the chemical composition and technological properties of meat, as established differences between the treatments were not statistically significant (P> 0.05) (*Ružić-Muslić,2006*). Our results are consistent with data of *Negovanović et al., (1983)*, who examined the impact of the level and the relationship between energy and protein in fattening lambs of Tsigai breed on meat quality, and concluded that

the applied nutrition treatment had no significant effect on the chemical properties of meat, considering that the share of water in the samples of *Musculus longissimus dorsi* (MLD) ranged from 75.29-76.28% and of protein 20.77-21.03%.

Table 3. Chemical and technological meat properties (*Ružić,1997*)

	Energy levels, MJ NEM		
	7.29	7.71 7.71 7.94	7.94
	Chemical composition,%		
Water	73.87±0.40	73.29±1.27	73.59±1.16
Proteins	21.5 ±0.80	22.05±0.47	22.19±1.05
Fat	3.51 ±0.80	3.57±1.31	3.02±1.22
Minerals	1.04 ± 0.02	1.06±0.06	1.05±0.04
	Technological properties,%		
Cooking loss	25.3 ±1.78	25.73±1.03	25.86±1.33
Roasting loss	35.95±1.58	34.63 ±3.73	35.22±2.08

Dutta et al. (2009) suggest that different levels of protein (12 and 14%) and energy (1.98 and 2.16ME Mcal/kg DM) in diets for fattening Barbari goats, did not affect the chemical composition of the meat, as there were no significant differences in moisture content, protein, fat and ash content between samples. Several studies have also shown that the chemical properties are independent of the influence of energy and protein level in the diet (*Craddock et al., 1974; Agnihotri et al., 2006; Abdullah and Musallam, 2007*). The level of non-degradable protein in diets for fattening lambs (43 : 51 : 58%) had significant influence on chemical and technological properties of meat, since the amount of water in the investigated MLD samples was: 75.11 : 75.0 : 75.0%. Protein content ranged from 21.46 : 21.62 : 21.77%, while the intramuscular fat content was: 2.28 : 2.16 : 2.12% (*Ružić-Muslić, 2006*). The content of intramuscular fat tissue is associated with the expression of specific sensory properties as well as better culinary quality of meat. *Eric et al., (2003)* state that the content of 2-3% intramuscular fat in muscle is sufficient for acceptable juiciness/succulence and tenderness of meat. When it comes to meat tenderness, the same authors state that it can be changed using a certain type of dietary fat (an important role in it have omega-3 fatty acids) and affect the diaphragm muscle in this way. Flax seed oil contains 60% omega-3 fatty acids and significantly contributes much softer and more tender meat/beef from cattle that were fed with a diet containing linseed oil, compared to meat from cattle that consumed the canola meal (containing 8 -10% omega-3 fatty acids, *Scollan et al., 2001*). So through feeding lambs, the structure and fatty acid composition of

lamb meat can be modeled to increase tenderness and content of polyunsaturated fatty acids in fat and muscle tissue of lambs.

Conclusion

Energy or protein deficinacy in the diet prevent full utilization of the genetic potential of lambs. In order to maximize utilization of the genetic potential of high yielding breeds of sheep for meat, in addition to the energy : protein ratio, the level of protein non-degradable at the rumen level is very important.

Nutrients whose proteins pass through reticulum-rumen and reach the duodenum to a greater extent non-degradaed, cause greater weight gain in lambs, in the presence of sufficient energy

Increasing the energy level of isoprotein diets results in an improved growth performance and better feed conversion. However, feeding lambs with a high concentration of energy causes acidosis, which sets new requirements for the ratio of carbohydrate components as well as energy and protein in the diet of fattening lambs.

Different levels of energy and protein in the diet of lambs have no significant impact on the dressing percentage, the yield of meat per categories, morphological composition of carcass sides and chemical and technological properties of meat.

Optimal balance between energy and protein in the diet of lambs optimizes microbial protein synthesis and maximum retention of degradable nitrogen in the rumen and thus affect growth performance.

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Optimizacija nivoa energije i proteina u obrocima za tov jagnjadi

D. Ružić-Muslić, M. P. Petrović, M. M. Petrović, Z. Bijelić, V. Caro Petrović, V. Pantelić, P. Perišić

Rezime

Ovce imaju sposobnost da transformišu hranu relativno niskog kvaliteta u visokokvalitetne proteine animalnog porekla (meso, mleko). Kako bi se povećala efikasnost iskorišćavanja hrane i minimiziralo zagađenje sredine, neophodno je bolje razumevanje potreba jagnjadi u nutrijentima(pre svega u energiji i proteinu), kao i njihovim interakcijama u organizmu.

Optimalan odnos između energije i proteina u obroku jagnjadi optimizuje mikrobiološku sintezu proteina i maksimalno zadržavanje razgradivog azota u buragu.Suvišna količina konzumirane energije u obroku, biće transformisana u mast, obzirom da je stepen porasta mišića i kostiju ograničen.Ishrana jagnjadi, deficitarna u pogledu proteina, dovodi do stvaranja velikih količina masti u organizmu. Opadanje efikasnosti korišćenja energije, pri suficitu proteina u obroku, dovodi se u vezu sa potrošnjom energije za formiranje i ekskreciju uree.

Imajući u vidu navedene činjenice, cilj ovog rada je da prikaže naše, kao i rezultate drugih autora, koji se odnose na optimizaciju nivoa energije i proteina u obrocima za tov jagnjadi.

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FATTENING ABILITY, CARCASS AND MEAT QUALITY AND RELATIONSHIPS BETWEEN ULTRASOUND MEASUREMENTS AND CARCASS QUALITY IN HEAVY LAMBS

P. Polák, J. Tomka, M. Margetín, D. Apolen, M. Gondeková, A. Oravcová, Z. Krupová

Animal Production Research Centre Nitra, Hlohovská 2, 951 41 Lužianky, Slovakia,
Corresponding author: polak@cvzv.sk
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Abstract: Fattening, carcass quality characteristics and ultrasound measurements of 40 heavy lambs of Tsigai and Ile de France breeds were analysed in order to judge possibilities of ultrasound measurements for *in vivo* assessment of meat production traits. Sonograms of transversal cut of *M. longissimus thoracis et lumborum* (MLTL) were obtained just before slaughter on last *thoracic vertebra* by echocamera Aloka PS 2 and ultrasound probe UST 5820 - 5. Muscle width, thickness and area and fat thickness were measured on digitalised sonograms in special software for video image analysis NIS - Elements. Tsigai lambs had significantly higher slaughter age, weight and muscle width. However Ile de France lambs had significantly higher muscle thickness, weight of meat in shoulder or proportion of round in half carcass. It means that here is significant difference between carcass composition between Tsigai and Ile de France. Lambs of specialised meat breed Ile de France in lower age and slaughter weight showed better meat production in comparison to Tsigai - dual purpose one. When we used ultrasound measurements with combination of daily gain or live weight before slaughter, coefficients of determination of the models were on level 0.09 - 0.24. Muscle width and daily gain had significant coefficient of correlation with carcass weight but the highest coefficient of determination for carcass weight was found in combination of live weight and subcutaneous fat layer (0.24). Despite of limited number of animals and quite large difference between carcass compositions of analysed breeds in this preliminary study, the findings indicate that ultrasound can be successfully utilized for *in vivo* assessment of carcass weight.

Key words: Heavy lambs, ultrasound, carcass quality

Introduction

Currently sheep production is being one of very limited number of growing commodities in Slovak animal production. It is not random that second one is suckler cow production and meat sheep production. All commodities are closely connected to using natural sources of green matter by grazing. It is maybe due to quite suitable geographical and climatic conditions. More than 60 % of Slovak territory is hilly and mountainous area with high level of steepness, high latitude, less fertile soil and high rainfall. Here are located more than 800 thousand hectares of natural meadows and pastures. Sheep production is one of very traditional ways of animal production of mentioned hilly and mountainous regions. There are two traditional breeds of sheep in Slovakia: Valachian and Tsigai sheep. Traditional product of dairy sheep is fresh cheese from which the national speciality "Slovenská bryndza" – PDI recognised product – is made. Tsigai is a dual purpose breed kept in traditional milk production scheme named "Carpatian system" with lambing in winter and selling of light lambs before Easter. Breeding season is accommodated to the date of next year Easter, because majority of lambs should be sold at this event, at about 12 kg of live weight. After selling of lambs, milking period starts and cheese is produced. Ewes are kept in mountain cottages named "salaš" during summer season. Production of heavy lambs by sucking their mothers on pastures is not traditional for the breed in the region, but due to various impact as diversification of production, market demands, high price of specialised meat production breeding stock and lack of trained work power is going to be more interesting today.

The aim of the investigation was to compare fattening ability, carcass and consumer meat quality from purebred Tsigai and Ile de France heavy lambs produced in ewe-lamb production system by sucking their mothers and natural grazing. Second aim was to analyse relationships among muscle measurements done by ultrasound on live animals and characteristics of carcass and meat quality.

Material and methods

Fattening characteristics, carcass and meat quality of 20 heavy lambs of Tsigai sheep (T) and 20 heavy lambs of Ile de France (IdF) were obtained at the average age of 137 and 106 days, respectively. Sonograms of transversal cut of *musculus longissimus thoracis et lumborum* (MLTL) were obtained just before slaughter on last *thoracic vertebra* by echocamera Aloka PS 2 and ultrasound probe UST 5820 - 5. Muscle width, thickness and area and fat thickness were measured on digitalised sonograms in special software for video image analysis NIS - Elements. Detail dissection of right half carcass was done 24 hours after

slaughtering to obtain weight and proportion of basic tissues (muscle, fat and bones). Weight of valuable cuts was calculated as sum of weight of round (boneless round without back shank), shoulder (boneless shoulder without front shank), back (MLTL between 1st *thoracic vertebra* and last *lumbar vertebra*) and tender loin. Samples (approximately 500 g) were taken from MLTL and for the first time they were analysed as fresh meat for physico-chemical meat quality after 48 hours of chilling. Samples of lamb's meat were processed before the analysis (removal of surface fat, membranes and tendons) and grounded. Chemical parameters of meat (proteins, fat and total water content) were analysed afterwards, when no more changes in chemical composition of meat are in progress. The apparatus Infratec 1265 with application module for fat content assessment 1 – 10 % was used. Seven days after slaughter, when boneless meat is supposed to be in consumer maturity, samples were cut into approximately 2 cm slices and were grilled afterwards. Smaller samples, so typical for lamb's meat, were left untouched, grilled in one piece. Contact grill PM – 1015 was used for samples processing. After 4 minutes, grilling losses and shear force of grilled meat were measured by Texture Analyser. Grilling losses were valued by weighing the samples before and after grilling. Basic statistics of all obtained variables and correlation coefficients between selected characteristics of carcass and meat quality were calculated. Statistical difference between two analysed groups of lambs was done by scheffe test. Linear regression models for weight of carcass and All analyses were done in SAS 9.2 statistical package.

Results and discussion

Tsigai lambs had significantly higher slaughter age, weight and muscle width (Table 1). However Ile de France lambs had significantly higher muscle thickness, weight of meat in shoulder or proportion of round in half carcass. It means that here is significant difference between carcass composition between Tsigai and Ile de France. Lambs of specialised meat breed Ile de France in lower age and slaughter weight showed better meat production in comparison to Tsigai - dual purpose one. Group of animals were highly statistically different in age of slaughter and live weight before slaughter. This fact affected our results very visibly. Nevertheless that carcass weight was higher in Tsigai lambs weight and portion of round and shoulder is higher in group of Ile de France. Relatively high variability, more than 10 %, were found for dressing percentage, proportion of leg in carcass in Tsigai breed (6.16; 7.35) where Ile de France recorded only 3.83 or 2.56 respectively. Both characteristics were 3 respectively 7 % higher in Ile de France. Here is visible difference between dual purpose and meat breed. Basic statistics of slaughter and carcass quality value are in Table 1 and 2. Characteristics

of consumer quality also showed high levels of variability in Tsigai breed then in Ile de France (Table 2). For example, shear force varied between 9.22 kg and 1.61 kg in T with average of 3.98 kg, what means that one piece of meat was very tender and other one was too tough. Meat of IdF was not significantly more tender with average value 3.39 but with smaller variability (1.10 – 5.17). For beef meat, suitable level is between 4 kg and 6 kg. It could be said that our sample of heavy lambs of both breeds obtained better results than those needed for cattle, even slightly higher than light lambs` meat. It is probably connected to the age of analysed lambs, which varied within 25 days, and live weight before slaughter (Table 1). In our opinion, we should focus on intramuscular fat level, level of daily gain and body/carcass conformation in the future.

Table 1 Basic statistics and statistical significations of slaughter value of 20 Tsigai and 20 Ile de France heavy lambs

Variable	Tsigai				Ile de France				Statistical importance
	Mean	Standard deviation	Minimum	Maximum	Mean	Standard deviation	Minimum	Maximum	
Live weight at slaughter	32,55	2,93	29,00	42,00	27,28	3,08	22,50	34,00	+++
Age at slaughter	137,40	8,27	123,00	151,00	106,95	8,20	95,00	117,00	+++
Cold carcass weight	12,32	1,54	9,70	17,00	12,83	2,12	10,00	18,00	+++
Dressing percentage	43,95	6,16	35,59	60,00	46,86	3,83	39,22	53,70	-
Average daily gain	237,66	24,79	207,14	289,66	256,26	34,05	221,24	343,75	+

Table 2 Basic statistics and statistical significations of carcass and meat quality and of 20 Tsigai and 20 Ile de France heavy lambs

Variable	Tsigai				Ile de France				Statistical importance
	Mean	Standard deviation	Minimum	Maximum	Mean	Standard deviation	Minimum	Maximum	
Dressing percentage	43,95	6,16	35,59	60,00	46,86	3,83	39,22	53,70	-
Weight of half carcass	6,14	1,30	1,47	8,38	6,58	0,90	5,20	8,70	+++
Portion of leg	27,25	7,35	2,48	31,40	34,97	2,56	29,35	39,65	++
Weight of shoulder	0,68	0,15	0,27	1,03	0,93	0,14	0,69	1,27	+++
Intramuscular fat portion	2,12	0,65	1,00	3,20	1,93	1,26	0,50	5,40	-
Warner Bratzler shear force	4,01	1,99	1,61	9,22	3,39	1,05	1,10	5,17	-

Table 3 Basic statistics and statistical significations of ultrasound measurements of 20 Tsigai and 20 Ile de France heavy lambs

Variable	Tsigai				Ile de France				Statistical importance
	Mean	Standard deviation	Minimum	Maximum	Mean	Standard deviation	Minimum	Maximum	
Width of MLD	5,90	0,50	4,62	6,73	5,02	0,44	4,15	5,74	+++
Height of MLD	2,04	0,19	1,73	2,39	2,29	1,34	1,72	7,95	-
Fat thickness above MLD	0,30	0,05	0,23	0,41	0,34	0,05	0,22	0,42	++
Area of MLD	7,79	1,41	5,41	11,15	6,77	0,96	5,29	8,60	+

Table 4 Pearson correlation coefficients between measured characteristics without respect of breeds.

	Age at slaughter	Cold carcass weight	Dressing percentage	Weight of half carcass	Portion of leg	Weight of shoulder	Width of MLD	Height of MLD	Fat thickness above MLD	Area of MLD	Intramuscular fat portion	Warner Bratzler shear force	Average daily gain
Live weight at slaughter	**0,62**	0,29	-0,17	0,15	**-0,46**	-0,25	**0,46**	-0,23	-0,12	**0,46**	0,07	**0,39**	**0,36**
Age at slaughter		-0,05	-0,22	-0,22	**-0,64**	**-0,62**	**0,58**	-0,20	**-0,51**	**0,37**	0,04	**0,36**	**-0,49**
Cold carcass weight			**0,49**	**0,75**	0,01	**0,64**	-0,02	-0,22	**0,36**	0,02	-0,10	0,04	**0,40**
Dressing percentage				**0,45**	0,18	**0,38**	0,03	-0,07	0,25	-0,02	-0,07	0,02	0,07
Weight of half carcass					**0,46**	**0,73**	-0,08	-0,18	**0,35**	-0,09	-0,14	-0,10	**0,41**
Portion of leg						**0,54**	**-0,38**	0,14	**0,31**	**-0,41**	0,01	**-0,35**	0,19
Weight of shoulder							**-0,38**	0,03	**0,42**	-0,21	**-0,20**	-0,19	**0,46**
Width of MLD								0,01	**-0,27**	**0,65**	-0,10	0,17	-0,19
Height of MLD									0,01	0,03	0,19	0,01	-0,04
Fat thickness above MLD										-0,06	0,04	-0,08	0**,48**
Area of MLD											-0,28	0,29	0,05
Intramuscular fat portion												0,09	0,02
Warver Bratzler shear force													-0,06

It is very difficult to compare our results with results of other authors due to differences in breeds, sex, and age at slaughter or fattening strategies *(Dickerson et al., 1972; Salomon et al., 1980; Notter et al., 1991; Ochodnický et al., 1994; Brady et al., 2003; Teixeira et al.,* 2006, *Daraban, 2008; Santos et al., 2008)*. Those authors, who analysed younger lambs *(Ghita et al., 2009)*, lighter ones refer results about quality of very young lamb meat. As a conclusion, it is possible to say that water content was higher and content of basic organic matter in muscle was lower. Also shear force was lower for younger lamb meat. *Apolen et al. (2002)* refer similar results of carcass quality in their work; the only exception was fat content in carcass, which was slightly higher. It is because they worked on similar genotype; the only difference was the system of fattening. They applied common fattening based on conserved roughage and concentrates. Ultrasound measurements showed difference in width of MLTL which was statistically significant higher for Tsigai lambs. On the other hand, height of MLTL was slightly higher for Ile de France lambs. Results of ultrasound measurements should be just expression of different age of slaughter, because it is known that in lambs legs, muscles in legs growth rapidly than middle of the back. It must be said that 30 days in live time of lambs is high difference. In 30 days Ile de France lambs should gain near 8 kg of live weight. Structure of carcass also should change in increasing age and weight.

When we used ultrasound measurements with combination of daily gain or live weight before slaughter, coefficients of determination of the models were on level 0.09 - 0.24. Muscle width and daily gain had significant coefficient of correlation with carcass weight but the highest coefficient of determination for carcass weight was found in combination of live weight and subcutaneous fat layer (0.24).

Conclusion

Despite of limited number of analysed animals in this preliminary study, the findings indicate that dual purpose breed Tsigai in ewe – lamb production system is able to produce heavy lambs with good meat quality. Ile de France breed special for meat production produces heavy lambs with higher quality than Tsigai dual purpose one. Results also showed that there is high variability of carcass and meat production traits within group of purebred Tsigai animals. Concerning to the using ultrasound we did not find significant results but coefficients of correlation shoved relationship between backfat thickness and muscle area with characteristics of carcass quality. In our opinion, we should increase number of observations and focus on intramuscular fat level, level of daily gain and body/carcass conformation in the future.

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Tovna sposobnost, kvalitet polutki i mesa i odnos između ultrazvučnog merenja i kvaliteta trupa teških jagnjadi

P. Polák, J. Tomka, M. Margetín, D. Apolen, M. Gondeková, A. Oravcová, Z. Krupová

Rezime

Tovnost, karakteristike kvaliteta trupa i ultrazvučna merenja 40 teških jagnjadi cigaja i il de frans rase su analizirani u cilju procene mogućnosti ultrazvučnih merenja za in vivo procenu osobina mesa. Sonogrami poprečnog preseka *M. longissimus thoracis et lumborum* (MLTL) dobijeni su neposredno pre klanja na poslednjem torakalnom pršljenu korišćenjem eho-kamere Aloka PS 2 i ultrazvučne sonda UST 5820 - 5. Širina mišića, debljina i površina, kao i debljina masnog tkiva su mereni na digitalizovanom sonogramu specijalnog softvera za analizu video snimka NIS - Elements. Cigaja jagnjad su imala beći uzrast pred klanje, kao i težinu i širinu mišića. Međutim, il de frans jagnjad su imala znatno veću mišićnu masu, debljinu mesa u plećki odnosno odnos udeo buta u polutki trupa. To znači da je ovde utvrđena značajna razlika u sastavu polutki između rasa cigaja i il de frans. Jagnjad specijalizovane mesnate rase il de frans, u nižem uzrastu i manjim težinama pred klanje pokazala su bolju mesnatost u odnosu na grla rase cigaja – rasa za dvostruku namenu. Kada se koristi ultrazvučno merenje sa kombinacijom dnevnog prirasta ili žive mase pre klanja, koeficijenti determinacije u okviru modela bili su na nivou od 0,09 - 0,24. Širina mišića i dnevni prirast imali su značajan koeficijenta korelacije sa težinom trupa, ali najviši koeficijent determinacije za težinu trupa je utvrđen u kombinaciji žive mase i potkožnog masnog tkiva (0.24). Uprkos ograničenog broja životinja i prilično velikih razlika između sastava trupa analiziranih rasa, u ovoj preliminarnoj studiji, rezultati ispitivanja pokazuju da se ultrazvuk može uspešno koristiti za *in vivo* procenu težine trupa.

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THE ATTITUDE OF SLOVENIAN FARMERS TOWARDS WOLVES

M. Kastelic[1,2], D. van Liere[3], A. Premik Banič[4], N. Siard[1]

[1] University of Ljubljana, Biotechnical faculty, Department of Animal Science, Groblje 3, 1230 Domžale, Slovenia
[2] Jata – Emona, Agrokombinatska 84, 1000 Ljubljana, Slovenia
[3] CABWIM consultancy, Minervalaan 17, 9401 EB Assen, Netherlands
[4] Educational Society Noah School, Založka cesta 155, 1000 Ljubljana, Slovenia
Corresponding author: miran.kastelic@jata-emona.si
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Abstract: The southern part of Slovenia is habitat of wolf, a protected species, and traditionally sheep farming area. Wolf attacks on sheep are a constant problem in the area, therefore attitude of sheep breeders towards wolves is very important and was studied as part of a project "Comparison of farms as a tool to prevent wolves from attacking sheep and to improve coexistence in rural areas". Sixty sheep farmers from the area were asked about their opinion on wolves, half of them had experience with wolf attacks in the period 2008-2010 (group A) and half of them did not (group NA). Two thirds of breeders or more from both groups expressed the following opinions: wolves are not endangering people, there is enough wild prey for wolves, reductions in wolf numbers would reduce the number of attacks on sheep, every wolf which approaches man or sheep should be killed and presence of wolves does not have touristic value. Around half of the breeders from both groups believe that coexistence with wolves can be improved by having good knowledge of wolves and that the state should solve problems with wolf depredation also in other ways, besides shooting and payment for damages. The opinions of groups were not statistically significantly different. Only exceptions were answers on question "Is the number of attacks related to the number of wolves?" Here 73 % of farmers from the A group and 78 % from the NA group answered with "yes", but the rest from the first group answered with "no opinion" and from the other group, with one exception, with "no" ($p=0.006$). In all, experience with wolf attacks had little influence on attitudes of farmers towards wolves.

Key words: wolf, sheep, attacks, attitude

Introduction

Wolf is a protected species in Slovenia, and its habitat in the southern part of the state overlaps with sheep breeding areas, where sheep are traditionally kept. The soil is low-productive and shallow and therefore the area is not suitable for modern intensive farming with cattle. In addition, high plant biodiversity is

maintained by sheep grazing. Without sheep farming the landscape would be overgrown with trees in the next two or three decades.

Tradition of sheep farming was interrupted during the last century. In that period the population of sheep and the number of wolf attacks were small. The number of sheep increased again during the last two or three decades as a result of natural conditions and agricultural politics. Wolf numbers have only been very roughly estimated and it is hard to tell whether there has been an increase or decrease. Although the state compensates the damage, the farmers are not satisfied. This is because the damage done is not only immediate (loss of sheep), but can also be long-term, from stressed, surviving animals producing less. Furthermore, sheep welfare is heavily compromised.

Attitudes of different social and occupational groups towards wolves are usually studied in the states inhabited by wolves. Wolves are generally regarded as superior, charismatic species (*Figari and Skogen, 2011*). However, attitudes of people towards wolves and large carnivores in general are complex, combining beliefs, feelings and knowledge (*Glikman et al., 2012*). Slovene research on attitudes towards wolves and wolf management among breeders of small ruminants, hunters and general public, showed that more than half of respondents supported conservation of wolves. Lowest support was expressed by breeders of small ruminants and half of the respondents believed that the damage caused on small ruminants is intolerable (*Marinko and Majić Skrbinšek, 2011*).

In Slovenia, sheep and wolves will continue to share the same area in the future and since livestock depredation is main cause of conflict, attitude of sheep breeders is very important in maintaining wolf preservation. Among sheep breeders, experience with attacks might be an important factor in expressed opinions. They were studied as part of a project "Comparison of farms as a tool to prevent wolves from attacking sheep and to improve coexistence in rural areas" (*van Liere et al., 2011; 2013*). Attitudes were researched in the period 2010-2011, at the point when attacks on small ruminants increased nine fold and compensations paid by the government increased tenfold in the period of 5 years (2005-2010) *(ARSO, 2005-2010*).

Material and Methods

Sixty farmers, half of them with experience of wolf attacks (A) on their flocks in the years 2008-2010 and half of them without experience with attacks in that period (NA) were asked on their attitude towards coexistence of wolves and sheep farming. They answered the following eight questions (Q1 to Q8):

1. Would people be in danger, where attacks on sheep occur?

2. Is the number of attacks related to the number of wolves?
3. Do wolves attack sheep, because there is not enough wild prey?
4. Would reduction in wolf number reduce number of attacks on sheep?
5. Should every wolf which approaches man or sheep be killed?
6. Would coexistence with wolves be improved by having good knowledge of wolves?
7. Should the state solve problems with wolf attacks in other ways, besides shooting and payment for damages?
8. Is the presence of wolves potentially interesting for tourism?

Possible answers were "yes", "no" or "no opinion". With "no opinion" breeders were expressing hesitation. Because some farmers did not answer all questions, the number of answers per question differed. The 2 x 3 contingency tables (experience of attack ("yes", "no"), three possible opinions ("yes", "no", "no opinion")) were constructed. The homogeneity of contingency tables, i.e. the similarity of distributions of answers between groups A and NA was tested with Fisher exact test which is a good choice in experiments with low number of measurements. Statistical evaluation and figures were done with statistical package R, ver.3.0.0.

Results and Discussion

The frequencies and percentages of opinions on questions and the results of Fisher exact tests according to previous experience with wolf attack are presented in Figure 1. Most farmers, around 70 %, independent of experience with attacks, believe that wolves are not dangerous for people (Q1). A comparable opinion was observed in Norway (*Figari and Skogen, 2011*). In Slovenia, general public and breeders of small ruminants were a bit reserved on that issue (*Marinko and Majić Skrbinšek, 2011*).

Most of the breeders, 73.08 % from group A and 77.78 % from the other group believe that the number of wolves is correlated with the frequency of attacks on sheep (Q2). However, in NA group nearly all of the rest of the breeders stated a clear opposite opinion, while only a few (3.70 %) were unclear in their position. In group A the situation was reversed, close to 27 % of the breeders had no opinion and there was no breeder who would clearly state there is no correlation between the number of wolves and frequency of attacks. The difference between the groups is statistically significant. Most of the breeders from both groups believe there is enough wild prey for wolves (Q3). The experience with wolves did not influence the opinion of the breeders. Three quarters of the farmers from group A and two thirds from group NA believe that the reduction of wolf population would decrease the number of attacks (Q4). The percentage of non-decided is the same in both groups, which is a bit surprising. Comparable answers would be expected as in Q2, since the questions are related. The differences between groups were not confirmed

with Fisher's exact test for Q4. In the research of *Marinko and Majić Skrbinšek (2011)* majority of Slovene breeders believe the numbers of wolves are too high.

Most breeders (88.89 % from group A and 62.96 % from group NA) answered that wolves, which are found in proximity of man or/and sheep must be shot (Q5). Only 7.41 % from group A share a belief that killing of such animals is not necessary. The same opinion had 29.63% breeders from NA group. The probability of Fisher exact test is 0.067, which is very close to statistical significance (P< 0.05). It is expected that farmers who experienced attacks would be more in favour of eliminating wolves which present a threat. In the research of *Marinko and Majić Skrbinšek (2011)* a vast majority of breeders support elimination of attacking wolves.

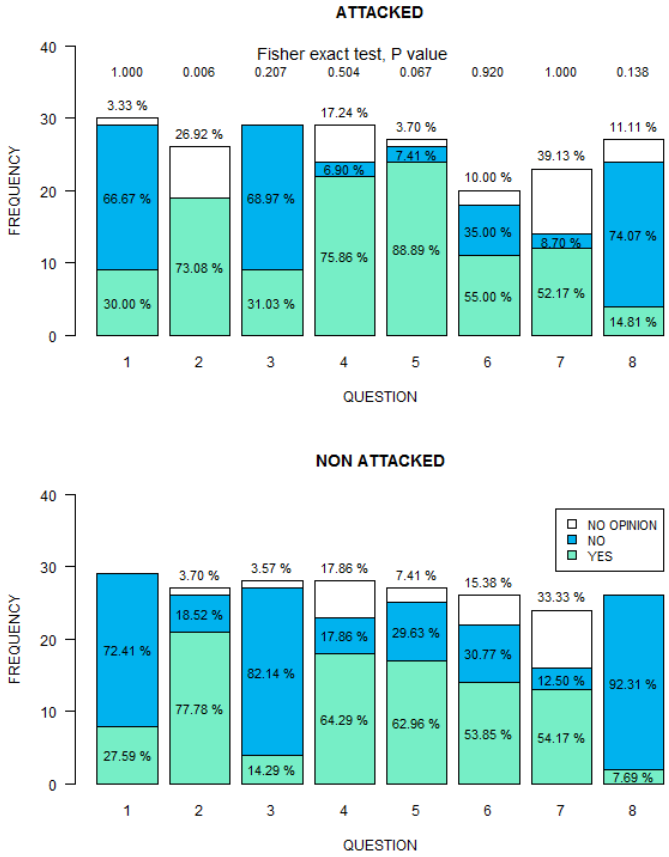

Figure 1. Frequencies and percentages of answers by farmers divided into groups with experience of wolf attack (attacked) and without experience of attack (non attacked) in period 2008-2010. The questions are numbered as in Material and methods. The P-values from Fisher exact test are presented on top of the first graph ("ATTACKED")

Experience with wolf attacks did not influence answers on the last three questions. A little more than half of the breeders from both groups believe that coexistence can be improved with more knowledge about wolves (Q6) and that the state can solve problems with wolf attacks also in other ways, besides shooting and payment for damages (Q7). Around one third of the breeders from both groups did not suppose that knowledge would improve coexistence, while more than one third of the breeders could not decide if there are any effective measures for reducing damages beside shooting and payment. The role of the state in damage prevention was strongly expressed in the research of *Marinko and Majić Skrbinšek (2011)*.

Vast majority of breeders, 74.07 % from group A and 92.31 % from group NA do not believe that the presence of wolves is interesting for tourism (Q8). Comparable results are reported by *Marinko and Majić Skrbinšek (2011)*.

In all, experience of wolf attacks did not affect much the opinions of the farmers. This might reflect strongly shared cultural beliefs, norms and values in rural populations as suggested by *Chavez et al. (2005)*.

Conclusions

Wolves are not perceived as dangerous to people, not even among the farmers experiencing wolf attacks on their herds.

General belief that number of wolves is related with frequency of attacks was confirmed. However, experience with attacks affected the opinions of the farmers who didn´t believe there is a relation. Those with no attacks were clear there is no connection, while the attack group was hesitant in its opinion.

Farmers from the attack group are strongly in favour of shooting wolves that approach; the difference with no attack group was close to significance.

Vast majority of breeders share the opinion that availability of wild prey is high enough for the wolves. Moreover, they share the opinion that wolves are not a touristic attraction.

Around half of the breeders believe that knowledge can improve coexistence with wolves and that the state could solve the problem of attacks with other measures beside shooting and damage compensations.

In all, experience with wolf attacks had little influence on the opinion of farmers.

Odnos slovenačkih farmera prema vukovima

M. Kastelic, D. van Liere, A. Premik Banič, N. Siard

Rezime

Južni deo Slovenije je stanište vukova, zaštićene vrste, ali i tradicionalna ovčarska oblast. Broj ovaca povećan je u poslednje dve ili tri decenije, kao rezultat prirodnih uslova i poljoprivredne politike. Vlada je nadoknadila štetu od napada vuka, ali odgajivači nisu bili zadovoljni. Zemljište je nisko-produktivno i plitko i stoga prostor nije pogodan za modernu intenzivnu stočarsku proizvodnju. Biodiverzitet je visok i ovce na ispaši ga održavaju. Bez ovčarstva pejzaž bi bio obrastao drvećem u naredne dve ili tri decenije. Odnos odgajivača ovaca prema vukovima je stoga veoma važan, a ispitan je kao deo projekta "Poređenje farmi kao sredstva za sprečavanje napada vukova na ovce i da bi se poboljšala koegzistencija u ruralnim područjima". Šezdeset farmera ovaca su anketirani i zamoljeni da daju mišljenje o vukovima. Stada polovine farmera iskusila su napad vuka (grupa A) u periodu 2008-2010, a polovina njih nije (grupa NA). Mogući odgovori su bili "da", "ne" i "nemam mišljenje". Neki farmeri nisu odgovorili na sva pitanja. Broj odgovora bio je 26 do 30 po grupi. Rezultati, frekvencije odgovora, ocenjeni su sa Fišer testom. Oko dve trećine ili više odgajivača iz obe grupe, izrazilo je sledeće mišljenje: vukovi ne ugrožavaju ljude, ima dovoljno divljeg plena za vukove, smanjenjem broja vukova bi se smanjio broj napada na ovce, svaki vuk u blizini čoveka i ovce treba biti ubijen i prisustvo vukova nema turističku vrednost. Oko polovine od odgajivača iz obe grupe smatraju da se suživot sa vukovima može poboljšati kroz dobro poznavanje vukova i da država treba da reši probleme sa pustošenjem na druge načine, pored snimanja i isplatu štete. Mišljenja grupa nisu statistički različita. Jedini izuzetak su bili odgovori na pitanje "Da li je broj napada povezan sa brojem vukova?", Gde je 73% farmera iz A grupe i 78% iz grupe NA odgovorilo sa "da", ali je ostatak iz prve grupe odgovorio sa "nemam misljenje" i iz druge grupe, sa jednim izuzetkom, sa "ne" ($p = 0,006$). Sve u svemu, iskustvo sa napadima vukova imali malo uticaja na stavove farmera prema vukovima.

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FORAGE QUALITY, PRODUCTION AND CONSERVATION ON PERENNIAL GRASSES

D.Sokolović, B. Dinić, S. Babić, J. Radović, Z. Lugić, Z. Tomić, G. Jevtić

Institute for forage crops, Kruševac, 37251 Globoder
Corresponding author: dejan.sokolovic@ikbks.com
Invited review paper

Abstract: High quality of animal feed and especially herbage, have positive effect on profitability of milk and meat production and it is prerequisite for its improvement and reduction of costs in production. Perennial forage grasses, main components of natural and sown grasslands, are natural food for ruminants and they give volume, essential for good rumen function since they have high content of fibres. They also have considerable mineral and protein contents that cover a large part of animals' requirements. Grasses usually produce the majority of the forage ingested by ruminant animals during grazing season, while during winter staple housing grass hay and silage are often major parts of the diet. Grasses are above all a healthy food, usually free of residues or toxic substances and production of meat and milk based on them meet society's needs not only in terms of quantity and feeding value, but also considering absence of toxic substances, high organoleptic qualities and production according ethical rules acceptable to society. Most of perennial grasses and their contemporary cultivars are highly productive and reach yield over 13tha^{-1} of dry matter of excellent quality. Generally, most of perennial grasses cultivars show high forage digestibility, balanced NDF and ADF content and crude protein content over 140gkg^{-1} of dry matter. The most common way of forage grasses production is within mixtures with perennial legumes, since in that kind of production the highest quality herbage yield has been achieving and ratio between crude protein and energy in the biomass is balanced. The most dominant perennial grasses forage conservation procedure is still making of hay, even it is connected with great losses during manipulation of biomass. Silage making of perennial grasses, as way of conserving of ruminant feed, becomes more important, since in this way nutritional value of herbage has been maximally preserving and ensures stabile and sustainable production of meat and milk. Without silage, especially maize and grasses, serious and economical cattle production is inconceivable. High concentration of WSC, low BC and their favourable ratio give perennial grasses high suitability for ensiling and make their biomass appropriate for silage making with difficulty ensiling biomasses, like

legumes. Knowing the suitability for ensiling of different perennial forage species it is possible to create adequate grass-legume mixtures for ensiling with good nutritive composition of the biomass in the same time.

Key words: perennial grasses, grasslands, forage yield, quality, utilization, conservation, silage

Introduction

Increasing of food production and intensification of agriculture production nowadays impose itself as necessary requirement for contemporary mankind survival in two different reasons. First is rapid increasing of inhabitant number on globe and need for changing of structure and quality of diet, and second is decreasing of agricultural land due to urbanisation in low land or abandonment of villages (reduction of number of inhabitants) in hilly - mountainous region *(SYS, 2013)*. Improvement of animal feed production and animal husbandry (beef and sheep production), generally is interrelated with production on agriculture land in hilly - mountainous region (natural and sown meadows and pastures).

Quality of animal feed and especially herbage, affects the economy of milk and meat production and it is prerequisite for its improvement and reduction of costs in production. Since herbage participation in ruminant diet is high, its quality may reduce needs for expensive imported high protein nutrients *(Dinić et al. 1997*).

In Serbia there is lack of protein in herbage and in animal diet in general. Therefore, low production and high prices of milk and meat are mostly consequence of low herbage quality and high share of concentrated feeds in ruminant meal. These facts are confirmed by research of *Schneeberger (1987)* who achieved cow milk production of 4337-5902 kg with high quality herbage and using only 58 to 63g of concentrate per litre of produced milk. In Serbia usually 200 to 400 gkg^{-1} of concentrate has been using for 1 l of milk produced.

Since, the main role of grassland and forage grasses is to ensure a supply of livestock products as a source of food for domestic animals, they have strong contribution to rural agricultural and economic development of the state (*Stošić et al., 2005*). Grasses are a natural food for ruminants and if they are eaten at a sufficiently young stage, they are highly digestible and contain lot of energy. Since they are rich in fibre, they provide the bulk necessary for good rumen function. They also have high mineral and protein contents that cover a large part of animals' requirements. Grasses usually produce the majority of the forage ingested by ruminant animals during grazing season. During winter housing grass hay and silage are often major parts of the staple diets.

Low production and high prices of milk and meat are also consequence of insufficient implementation of contemporary technologies in production and conservation of herbage *(Lugić and Dinić, 2010)*. Hay making is still dominant way of herbage conservation in hilly mountainous region of Serbia and in most parts of Serbia it is of low quality in terms of nutrition content (low crude protein content and high concentration of crude fibre and lignin) due to great losses of quality biomass during drying *(Dinić et al., 2011a)*. Late movement in inadequate phase of grassland, unsuitable drying incorporated with unfavourable weather conditions and old mechanisation are reasons for low biomass quality, as well (*Dinić et al., 2002, Đorđević et al., 2011)*.

In many countries with developed agriculture, silage making is dominant way of conservation of grass and other herbage. For instance, in Germany hay has been making in amount of 2 million tons, while silage of grasses is preparing in amount four times higher (8.6 million tons) *(Wilkinson and Toivonen, 2003)*.

The aim of this paper is to specify importance of perennial grasses and grasslands as resources in hilly mountainous region for animal feed production, as well as its productivity, biomass quality and the best way of grass herbage conservation.

Grasslands and perennial forage grasses importance and productivity

Grasslands and grass vegetation in general occupies large areas of world's land area and represent largest ecosystems in the world (*Suttie et al., 2005*). This area under grasslands is estimated at 52.5 million km^2 or 40.5% of the total terrestrial areas, excluding Greenland and Antarctica. In Western Europe grasslands occupy almost 40% of the agricultural area while in some countries this share is even higher (Ireland 76%, Switzerland 72 %, United Kingdom 65% and Austria 57%). In Central and Eastern European countries grasslands are spread on more than 36% of agricultural area *(FAO, 2002)*. In Northwest part of EU grasslands meet 70 to 75% of the ruminant livestock population feed and 70 % of that grasses contribution comes from grazing (*Mayne et al., 2000*).

Since grasses represent crucial source of nutrients for livestock production and they allow the utilization of marginal soils that are unsuitable for arable cropping, they played a major role in the agricultural development in most parts of Europe during time. Nevertheless, recent decades, the area under the grassland was dramatically decreased, with a 25% reduction in France (*Huyghe, 2010*) mostly because of increase of abandoned areas and fields under annual forage crops. Due to abandonment of villages in upper regions of east and south Serbia permanent grassland are transformed in underbrush and forests. In Serbia natural grasslands cover large acreage over 1,45 million hectares, or almost 29% of total agriculture

land while fields under the sown grasslands are on 150.000ha (*Stošić and Lazarević, 2009*).

Perennial forage grasses represent biologically very complex and heterogeneous group of plants. On Balkan Peninsula and in Serbia large diversity of perennial grass species exists and many of them remained there in *refugio* after last ice age. They are acclimatized to change of seasons and different habitats from lowlands with fertile deep soil to mountainous terrains over 1200m a.s.l. with shallow soils and lack of nutrients and humus. Especially this broad adaptability to different agroecological and climatic conditions considerably increases agronomical importance of perennial grasses on the multiple bases, especially known the fact that they are the base components of grasslands and rangelands with more than 50% of share (*Vučković et al., 2005; Tomić et al., 2009*). Furthermore, they are cenobionts and edificators of whole series of plant associations on natural meadows (*Vučković, 2004; Tomić et al., 2005*). State of biodiversity of grasses and grasslands in Serbia is still on high level including some meadow plant communities (*Festuceto-Brometea*) consisted of species representing about 42% of total flora in Serbia (*Dajić-Stevanović et al., 2010*). Although all European grasslands are more or less modified by human activity and can be defined as "semi-natural", they have the best ecosystem quality of all possible agricultural production systems and provide undisturbed situation to species originally present in natural ecosystems (*Reidsma et al., 2006*). Therefore, preserving of biodiversity is one more contribution of perennial grasses and grasslands to environmental performance. Also, perennial grass ecosystems protect soil, water resources and natural habitat. Losses of nutrients and nitrate leaching may have very negative consequences on the groundwater quality and result in final in water pollution. Perennial grasses form dense cover with haired root system which preserves soil from water runoff and reduce nitrate leaching by efficient consumption (*Benoit and Simon, 2004*) and also improve hydrological cycle and water quality. The older grassland has higher infiltration capacity, better soil structure, more earthworm burrows and a higher organic matter content. Grasses perenniality also determines the exploitation regime and sward persistency. Infrequent need for renovation relaxes the soil and reduces a significant part of the losses during ploughing.

The intensification of forage production and utilization, especially during the second half of the twentieth century, led to a drastic reduction in the number of grass species in sown mixtures, as well as in permanent grasslands. Only a small number of species mainly the ryegrasses, timothy, cocksfoot and fescues are highly suited to intensive management. Moreover, in Europe from 1980 onwards, many forage systems were de-intensified and wild or hardly selected species became

more important in grasslands. Nevertheless, it is evident that importance of perennial grasses and grassland as animal feed will be increased in the future.

Productivity

The final aim of exploiting grasses in agriculture is transformation of herbage into livestock products. Since grasses and grasslands represent essential feed for ruminants, in Europe 50 to 75% of cattle and 90 to 95% of sheep fodder requirements are met by them on annual basis. Perennial grass leaves are broad and flexible enough to be easily grazed by cattle, sheep and goats and soft enough to be easily attacked by rumen microorganisms. Grass is above all a healthy food, usually free of residues or toxic substances. Produced meat and milk should not only meet society's needs in terms of quantity and feeding value, but should also be free of toxic substances, have high organoleptic qualities and be produced according to ethical rules acceptable to society. Livestock products based on the utilisation of grasses are in an excellent position to meet these conditions. In this way grasslands and rangelands give high contribution to economic development.

Most of perennial grasses and their contemporary cultivars are highly productive and reach yield over 13tha^{-1} of dry matter (DM) (*Peeters, 2004*; *Sokolović et al., 2010a*) of excellent quality. Italian ryegrass can reach 14 to 15.5 tha^{-1}, meadow fescue 11.5 tha^{-1} and tall fescue over 13 tha^{-1} of DM (*Tomic and Sokolovic, 2007)*.

Perennial ryegrass, the most important forage grass species on European level, is also highly productive rain-fed conditions in Serbia where can achieve in favourable years 14tha^{-1} of DM (*Sokolovic et al, 2010b)*. Regularly in average it can produce about 8.6 tha^{-1} of DM and 815 kg of crude protein per ha (*Sokolovic et al, 2007a)*, what easily may be improved with N fertilisation.

Cocksfoot, which is one of the most important perennial forage grasses in Serbia, may produce between 12 and 16 tha^{-1} of high quality DM (*Tomić et al., 1994; Tisserand, 2002, Sokolovic et al., 2004*) and 2.17 tha^{-1} of CP. Also timothy, in area beyond altitude of 600 to 800m a.s.l, can be very productive in Serbia with yield of about 10 tha^{-1} of DM in two or three cuts *(Tomić et al., 1997).* Within yield of 8.2 tha^{-1} of DM, it contributes with 1.34 tha^{-1} of CP *(Sokolovic et al., 2004)*.

Red fescue, which concerning quality belongs secondary group of fodder grasses, is persistent and resistant grass species which can achieve yield over 10tha^{-1} of DM *(Tomić et al., 1998)* or 1.33 tha^{-1} of crude protein *(Sokolovic et al., 2004)*. In favourable year best genotypes of red fescue in trials can produce 13.7tha^{-1} DM *(Sokolovic et al., 2007b)*.

Tall oatgrass can give crude protein yield 1028kgha^{-1} and more than 8tha^{-1} of DM (*Sokolovic et al., 2008)*.

DMY of perennial grasses is easy improvable by application of fertilisers, especially nitrogen (*Stošić et al., 2005*), nevertheless this can influence on floristic composition and reduction of legume component in grasslands which is important for symbiotic N fixation. It can reduce transfer of N from legumes to grasses in mixture and possible over-yielding (*Huyghe, 2010*) with potential risk of environment pollution by N leaching, as well.

Fodder quality of perennial grasses

Generally, forage of most of perennial grasses species shows high digestibility (*Wilkins, 1997*), balanced NDF and ADF content (*Casler, 2000*) and crude protein content over 140gkg^{-1} (*Tomić et al., 2007)*.

The most common way of grass cropping is within mixtures with perennial legumes, since in that kind of production the highest quality herbage yield has been achieving and ratio between crude protein and energy in the biomass is balanced better *(Dinić et al., 2000)*. Grass - legume mixtures vary less in yield compared to pure crops, during drying process have smaller losses and concerning fact that they have less needs in mineral fertilisers, economical effect of production is significantly higher. The most important long term effect of grass - legume mixtures is ecologically, since pollution of soil and underground water with reduced fertilisation and N leaching is decreased.

Chemical composition and nutrition value of perennial grasses biomass depend on several factors:

- Species and genotype

Significant differences in chemical composition and nutrition value are characteristic for different plant species in the same *genus* but also for different cultivars and hybrids in the same species. These differences as result of long lasting breeding and selection efforts by man have become more pronounced. Differences in cultivars or genotypes biomass quality can be very high (*Tomić et al., 2002; Babić et al., 2012; Sokolović et al., 2010b*), significantly affecting digestibility and usefulness as animal feed at final instance.

It is well known that tetraploid cultivars of Italian ryegrass have significantly more water soluble carbohydrates than diploids and concerning that they are much suitable for ensiling, therefore they can be grown in mixtures for ensiling with hardly ensiling biomasses as red clover or other legumes. Recent decades different interspecies hybrids were made for animal feed. In grasses the most important are Festulolium hybrids in which breeders succeed to combine positive traits of their parent species, persistency and drought tolerance of *Festuca*

pratensis and *F. arundinacea* on one side and biomass quality and digestibility of *Lolium perenne* and *L. multiflorum* on other side.

- Time of utilisation (growth stages)

Due to changes of plant tissue chemical composition during maturing it is essential with right cutting or grazing moment to adjust forage yield and nutritional value *(Anderson et al., 1994)*. Young plants contain higher share of leaf shields in relation to steams (Table 1), which is manifest in higher content of crude protein (CP) and minerals (Table 2). Their content has been decreasing with plant ageing while in the same time amount of NDF, ADF, fibres (CF) and lignin have been increasing.

Table 1. Leaf and steam ratio in plant (%) (*Dinić et al., 2002)*

Species	Growth stage			
	Beginning of tillering		Full tillering	
	Leaf	Steam	Leaf	Steam
Italian ryegrass	35.1	64.9	21.0	79.0
Perennial ryegrass	45.1	54.9	30.0	70.0

Table 2. Chemical composition of Italian and perennial ryegrass, gkg^{-1} SM *(Dinić et al., 2002)*

Biomass		CP	CF	Ash	P	Ca	Mg
Italian ryegrass							
Beginning of tillering	Fresh	131.4	338.1	142.1	3.2	4.5	3.2
	Wilted	135.0	334.3	128.8	3.1	4.9	3.2
Full tillering	Fresh	100.7	399.1	117.8	2.6	4.8	3.1
	Wilted	103.8	401.1	110.5	2.8	3.5	1.7
Perennial ryegrass							
Beginning of tillering	Fresh	164.2	329.2	163.6	5.0	6.0	7.5
	Wilted	172.2	327.0	134.9	3.8	5.9	5.7
Full tillering	Fresh	122.2	382.1	113.5	3.2	4.8	4.7
	Wilted	127.5	375.6	96.6	3.3	5.5	4.8
LSD 0.05		5.0	7.3	3.4	0.1	0.6	0.7
LSD 0.01		6.9	10.1	4.7	0.2	0.8	1.0

Maturation process enhances DMY in current vegetation cycle with simultaneous reduction of digestibility as consequence of intense process of lignifications of grass stems (*Buxton and Russel, 1988)*.

Orchardgrass, one of the most important perennial forage grasses with high quality biomass if it is cut in right moment, rapidly loosing its quality with maturity, especially in first cut (Table 3). High content of CP about 200 gkg^{-1} DM reflexes high quality biomass like in legumes. Using of legumes and grasses

biomass quality standards of American Forage and Grassland Council refer to content of CP in DM classify forage of orchardgrass in young stage in extraordinary quality biomass (above 190 gkg^{-1} DM).

Table 3. Chemical composition of orchardgrass biomass cv K-rana, gkg^{-1}DM (*Dinić et al., 2003)*

Growth phase	Beginning of heading		Beginning of flowering		LSD	
Chemical parameters	Fresh	Wilted	Fresh	Wilted	0.05	0.01
CP	190.5	205.3	176.4	182.6	10.7	16.2
CF	285.4	278.9	282.5	295.0	14.9	22.5
NFE	355.9	360.7	390.2	390.4	31.9	48.3
Ash	122.5	117.6	107.8	104.9	7.0	10.6
Ca	4.0	3.8	3.8	4.2	ns	
P	3.7	3.9	3.9	3.9	ns	
DM, gkg^{-1}	205.7	352.0	193.3	363.3	18.5	28.0

Due to differences in the time of maturity of different species, but also because there are cultivars with different maturity in same species, orchardgrass for instance *(Sokolović et al., 2011; 2012),* it is important to pay attention on earliness of genotypes in mixture composition to regulate best moment for utilisation of grassland and to obtain best quality forage.

Tall oatgrass (*Arrhenatherum elatius*) can give high quality biomass in pure stand (Table 4) if it is cut in the beginning of heading. But, it is generally early maturity species and therefore can make biomass of mixture where is present worst then it really is, since it is almost ripened in the phase when mixture is in the right time for cutting.

Table 4. Chemical composition of tall oatgrass biomass *(Dinić et al., 2008a)*

Chemical parameters	Heading phase	Flowering phase	Fresh biomass	Wilted biomass	LSD $_{0.05}$	LSD $_{0.01}$
DM, gkg^{-1}	308.3b	322.5a	233.8b	392.0a	4.37	6.63
CP, gkg^{-1} DM	156.0a	138.2b	144.2b	150.0a	2.91	4.40
EE, gkg^{-1} DM	50.05a	48.2a	49.3a	48.9 a	9.2	13.9
NDF, gkg^{-1} DM	690.1a	704.4a	711.7a	691.0a	19.51	29.51
ADF, gkg^{-1} DM	338.7a	340.3a	328.8a	335.2a	13.43	20.30
Hemicelluloses, gkg^{-1} DM	351.3a	364.1a	382.8a	355.8a	13.30	20.20
Ash, gkg^{-1} DM	104.4a	97.6b	102.8a	99.2a	4.69	7.10
Ca, gkg^{-1} DM	5.4a	4.9a	4.7b	5.6a	0.78	1.18
P, gkg^{-1} DM	3.53a	3.05b	3.33a	3.25a	0.27	0.40

[a,b] Values in the same row with different letters are statistically significantly different (P<0.05)

Red fescue (*Festuca rubra*), concerning quality secondary grass species, if it is cut in the beginning of tillering, show good quality forage with CP content above 150 gkg^{-1} DM (Table 5) and nutritive value in NE_L and NE_M units over 5 MJ kg^{-1} DM. This herbage quality is closely connected with plant development stage in the moment of cutting *(Dinić et al., 2011b).*

Table 5. Chemical composition and nutritive value of red fescue biomass

Nutritive values and energy of red fescue forage	
Dry matter gkg^{-1}	240
Crude protein, gkg^{-1}DM	153.3
Crude fibre gkg^{-1}DM	296.0
Ether extract gkg^{-1}DM	33.7
NFE gkg^{-1}DM	415.9
ASH gkg^{-1}DM	99.4
Ca gkg^{-1}DM	8.1
P gkg^{-1}DM	6.5
NE_L $MJkg^{-1}$DM	5.14
NE_M $MJkg^{-1}$DM	5.02

- Vegetation cycle

This factor influence dominantly on quantity and secondarily on fodder quality, mostly because changeable amount of precipitation during the year. In Serbia majority of rainfall falls in spring period of vegetation, therefore yield is relatively largest (%) in that period of the year. In further cuts yield of herbage has been decreasing and very often they are lowest in autumn period, when on plant yield influence have not only precipitation, but solar irradiation and temperature as well.

- Cropping management

This factor includes soil, climate and practice. From those prerequisite depend possibility of growing of certain species, quality and quantity of forage yield.

Also, hay quality depends on moving height, usage of conditioner, way of biomass drying, collecting and warehousing *(Đorđević et al., 1995; 2001).* Hay quality and nutritive value is under higher influence of weather conditions than species forming plant forage biomass (*Holmes, 1989).*

Forage grasses conservation by ensiling

Basic aim of different procedures for conserving grasses herbage for animal feed is maximal preserving of nutritional value as long as it is possible (*Đorđević et al., 2010*). Silage of perennial grasses as way of conserving of ruminant feed becomes more important, since ensues stabile and sustainable production of meat and milk. Without silage, especially maize and grasses, serious and economical cattle production is inconceivable.

Main prerequisite for successfully perennial forage grasses biomass ensiling, as for all other forages which are used for silage or haylage preparing, it is essential with certain procedures to provide anaerobic environment, optimal content of WSC and water in biomass.

Anaerobic environment is necessary for eliminating activity of numerous strains of mouldering microorganisms which need oxygen for their activity. In such conditions dominant are lactic fermentation bacteria which are facultative anaerobic and anaerobic microorganisms. With rapid providing anaerobic environment first oxidative phase is reducing and in the same way loses in nutritive substances in feed.

One more requirement for obtaining of quality and stabile grass silage is appropriate chemical composition of starting material and its suitability for ensiling. To know that, it is necessary to determine WSC and BC ratio as measure of potential biological acidification and DM content as measure of necessary degree of biological acidification.

Sugar content in biomass depend on species, cultivar, stage of development, plant part, climate and applied mineral fertiliser and therefore there are forage species which are ensiling easy (almost all perennial grasses), which are ensiling harder (herbage of red clover, forage pea, vetch, sainfoin and cocksfoot) and forage species which are extremely difficult to conserve as silage alone (alfalfa, soya bean, birdsfoot trefoil, white clover). Tetraploid cultivars contain more sugars than diploid cultivars, as well as mature plants in comparison with young one (*Dinić et al., 2002).* This lead to conclusion that is better to ensile older biomass, but this biomass shows inferior quality since it has less digestibility and nutrititive value.

Biomass of perennial grasses generally has favourable ratio between WSC and BC, and may supply other difficult to ensile biomasses with sugars. The ratio of WSC (gkg^{-1} DM) and BC (meq lactic acid in $100g^{-1}$ DM) may predict successful in conservation and if this ratio is higher than 3, silage would be stable and high quality *(Weissbach, 1967).* In research *Dinić et al. (2002*) suitability for ensiling of whole plant and some plant parts of perennial ryegrasses in different cutting plant stage is presented. It was determined that in both species ratio between sugars and BC is high and appropriate for ensiling, especially in stage of beginning of tillering when the biomass is better for animal feed since it contain more crude protein.

Table 6. Suitability for ensiling of fresh mass of stem, leaf and whole plant (WP) of Italian and perennial ryegrass *(Dinić et al., 2002)*

Biomass	DM gkg^{-1}	pH	Monosach. gkg^{-1} DM	Total sugars gkg^{-1} DM	BK, Meq LA 100g^{-1} DM	WSC/BK
Italian ryegrass						
Start of tillering – stem	172	5.10	285	314	11.63	27.00
Start of tillering – leaf	210	5.50	119	153	30.95	4.94
Start of tillering WP - fresh	140	5.77	242.0	308.6	28.09	10.99
Start of tillering WP - wilted	236.7	5.75	208.0	268.3	18.98	14.13
Full tillering – stem	244	5.58	193	234	12.30	19.02
Full tillering – leaf	198	5.89	121	149	20.20	7.38
Full tillering WP - fresh	188.3	6.15	190.3	250.6	22.15	11.31
Full tillering WP - wilted	350.0	6.39	119.3	140.7	23.14	6.08
Perennial ryegrass						
Start of tillering – stem	170	5.94	178	256	20.59	12.43
Start of tillering – leaf	180	5.86	136	189	33.33	5.67
Start of tillering WP - fresh	150.0	5.90	197.7	240.0	31.14	7.71
Start of tillering WP - wilted	363.3	5.93	118.7	145.3	24.73	5.88
Full tillering – stem	206	5.22	114	150	11.16	13.44
Full tillering – leaf	169	5.38	84	121	17.75	6.82
Full tillering WP - fresh	228.0	5.47	92.3	107.7	18.44	5.84
Full tillering WP - wilted	306.7	5.94	117.0	145.3	26.48	5.49
Lsd 0.05*	9.37	0.142	2.38	20.23	17.09	
Lsd 0.01*	13.01	0.198	3.31	28.08	23.72	

* LSD test is calculated only for parameters of whole plant biomass

High concentration of WSC, low BC and their favourable ratio (Table 7), give tall oatgrass high suitability for ensiling and make its biomass appropriate for ensiling with difficulty ensiling biomasses, like legumes.

Table 7. Suitability for ensiling of tall oatgrass biomass *(Dinić et al., 2008a)*

Chemical parameters	Heading stage	Flowering stage	Fresh biomass	Wilted biomass	LSD $_{0.05}$	LSD $_{0.01}$
DM, gkg^{-1}	308.3	322.5	233.8	392.0	4.37	6.63
Total sugars (S), gkg^{-1} DM	187.5	159.3	182.3	164.5	13.90	21.50
Buffer capacity (BK), meqv lactic acid 100g^{-1} DM	32.82	29.83	34.18	28.47	2.36	3.57
S/BK ratio	5.79	5.35	5.79	5.36	0.73	1.10

Knowing the suitability for ensiling of different perennial forage species it is possible to create adequate grass-legume mixtures for easy ensiling with good nutritive value in the same time.
Biomass of Italian ryegrass in comparison with red clover is more favourable for ensiling since has more sugars (Table 8), lower buffer capacity and consequently better ratio among them (*Dinić et al., 2000)*. It can be predicted based on those data that silage without butyric acid is possible to obtain directly only from biomass of Italian ryegrass and that red clover biomass should be wilted and to have more than 272 gkg^{-1} DM. Therefore, that could be the main reason for sowing of mixture of those two species, what is going to give biomass suitable for direct ensiling, balanced regarding content of protein and energy.

Table 8. Suitability for ensiling of Italian ryegrass and red clover *(Dinić et al., 2000)*

Biomass	Total sugars gkg^{-1} DM	BK, Meq LA $100g^{-1}$ DM	WSC/BK
Italian ryegrass	155	44.8	3.5
Red clover	131	58.8	2.23

It is already said that DM content of biomass is very important for ensiling and that spontaneous lactic fermentation has been happening and ensiling is happening only in biomasses with more than 25% of DM. For perennial grasses forage optimum water content is 70%, even it is changeable due to different WSC/BC ration. Wilting of biomass of above 40% of DM brings good material for making of haylage. As water content in biomass is lower, the pH of silage should be lower to have good quality silage. Higher DM content contribute adequate lactic fermentation and reduce content of undesirable products in silage like butyric acid and ammonium nitrogen *(Dinić et al., 1999)* which represent indicators of protein degradation and lower mark of silage for one or two class (*Dinić et al., 2003)*

Conclusions

High quality herbage as animal feed has positive effect on profitability of milk and meat production and it is prerequisite for its improvement and reduction of production costs. Perennial forage grasses, main components of natural and sown grasslands, are natural food for ruminants and they give volume essential for good rumen function since they have high content of fibres.
Most of the forage consumed by ruminant animals during vegetation season and winter staple housing comes from grass hay or silage. All perennial grasses and their contemporary cultivars are highly productive and reach yield over $13tha^{-1}$ of

dry matter of excellent quality. This high forage quality of grasses is reflected in high forage digestibility, balanced NDF and ADF content and crude protein content over 140gkg^{-1} of dry matter.

High concentration of WSC, low BC and their favourable ratio give perennial grasses high suitability for ensiling and make their biomass appropriate for ensiling with difficulty ensiling biomasses, like legumes. Knowledge the suitability for ensiling enables to create adequate grass-legume mixtures for ensiling with good nutritive composition of the biomass in the same time.

Silage making of perennial grasses, as way of conserving of ruminant feed, becomes more important, since in this way nutritional value of herbage has been maximally preserving and ensures stable and sustainable production of meat and milk.

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Kvalitet krmnih biljaka, proizvodnja i konzervacija višegodišnjih trava

D.Sokolović, B. Dinić, S. Babić, J. Radović, Z. Lugić, Z. Tomić, G. Jevtić

Rezime

Visoko kvalitetna stočna hrana, naročito kabasta stočna hrana, ima pozitivan uticaj na profitabilnost u proizvodnji mleka i mesa. Unapređenje proizvodnje kabaste stočne hrane je preduslov za smanjenje troškova u proizvodnji mleka i mesa. Višegodišnje krmne trave, kao glavne komponente prirodnih i sejanih travnjaka, su prirodna hrana za preživare. Imaju visok sadržaj vlakana što je bitno za dobru funkciju buraga preživara, kao i značajan sadržaj mineralnih materija i proteina, koje pokrivaju značajan deo potreba životinja u ovim materijama. Trave predstavljaju najzastupljeniju komponenatu kabaste hrane za preživare naročito tokom sezone ispaše, ali i tokom zime u obliku sena ili silaže kao glavni deo obroka. One su pre svega zdrava hrana, uglavnom bez rezidua toksičnih materija. Ishrana životinja za proizvodnju mleka i mesa bazirana na višegodišnjim travama zadovoljava potrebe društva ne samo u smislu količine i

kvaliteta već i etičkih normi s obzirom na odsustvo štetnih materija i visok orgaoleptički kvalitet krajnjih proizvoda.

Većina višegodišnjih trava kao i njihove savremene sorte su veoma produktivne i dostižu prinos od preko 13tha^{-1} suve materije odličnog kvaliteta. Generalno, većina sorti višegodišnjih trava ima visoku svarljivost suve materije, uravnotežen odnos ADF-a i NDF-a i sadržaj proteina preko 140gkg^{-1}.

Najčešći način proizvodnje krmnih trava je u smešama sa višegodišnjim leguminozama obzirom da takav način proizvodnje obezbeđuje najveći prinos i kvalitet biomase i izbalansiran odnos između sirovih proteina i energije. Najzastupljeniji način konzervisanja visegodišnjih trava je proizvodnja sena, iako su gubici tokom manipulacije biomasom na taj način najveći. Proizvodnja silaže od višegodišnjih trava, kao vid konzervisanja hrane za preživare, dobija na značaju, jer je na taj način hranljiva vrednost biomase najbolje očuvana i obezbeđuje se stabilna i održiva proizvodnja mleka i mesa. Bez silaže, posebno kukuruza i trava, ozbiljna i ekonomična stočarska proizvodnja je nezamisliva. Visok sadržaj vodorastvorljivih ugljenih hidrata i nizak puferni kapacitet, kao i njihov povoljan odnos čine trave pogodnim za siliranje bez teškoća koje se javljaju prilikom siliranja leguminoza. Poznavajući mogućnost siliranja različitih višegodišnjih krmnih vrsta moguće je stvoriti odgovarajuće travno-leguminozne smeše dobrog nutritivnog sastava u isto vreme.

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MEADOW MIXTURES IN SERBIA: CHALLENGES AND PERSPECTIVES

A. Simić[1*], Z. Tomić[2], S. Vučković[1], Z. Bijelić[2], V. Mandić[2]

[1] University of Belgrade, Faculty of Agriculture, 11080 Zemun-Belgrade, Serbia
[2] Institute for Animal Husbandry, Autoput 16, Belgrade 11080, Serbia
*Corresponding author: alsimic@agrif.bg.ac.rs
Invited paper

Abstract: The aim of this paper is to summarize some recent experiences and results in livestock feed production from sown meadows in Serbia with special emphasis being given to the importance of preserving legume species in mixtures and corresponding measures. Also, trials assessed the benefits of grass/legume mixtures in terms of forage production, testing whether the combination of different species improves the stability of the mixtures and evaluated response patterns over a large environmental gradient.

Key words: meadow, mixture, quality, yield

Introduction

Mixtures of two or more grass and legume crops are often more effective than planting a single species. Mixtures offer the best of both of families, combining the benefits of grasses and legumes, or using the different growth characteristics of several species to fit grower needs. Benefits of mixtures may include better use of solar energy, larger production of biomass and N production, tolerance of adverse conditions, as well as, better response to variable soil traits. Grass/legume mixtures are preferred over pure-grass forage stands throughout the world, because they often increase the total yields of herbage and protein and offer balanced nutrition *(Albayrak et al., 2011)*. Also, they offer several potential advantages over pure grasses or pure legumes, including the control of erosion, prolonged stand longevity and, especially, weed control.

Modern trends in Europe promote grass/clover mixtures to produce more farm grown proteins and to decrease the use of mineral fertilizers *(De Vliegher and Carlier, 2008)*. Growing animals have a high protein requirement that can be met to a large degree by adequate legumes in the forage mix. Furthermore, the palatability and digestibility may also be improved. Although planting a legume with a grass does not mean all nitrogen fertilizer needs will be met, grasses can

eventually benefit from being planted in soil where legumes fixed atmospheric nitrogen. As a rule, grass/legume mixtures are more productive than pure legume in the same way as grass species fertilized by 100 kg N *(Lazarević et al., 2005)*.

Serbian case study

The Republic of Serbia has significant natural resources and capacity (agricultural land, air, water, etc.), including agricultural population, livestock population, manufacturing and processing facilities and techniques, developed educational scientific activities, etc. The current level of livestock production in Serbia does not provide cost-effectiveness, therefore it is necessary to work more efficiently and to change the same organization to enhance capacity building in qualitative and quantitative terms. In the structure of agricultural production in Serbia, about 65% derives from the plant production, and less than 35% is livestock production (EU - 70% livestock production and 30% of crop production) (*Petrović et al.,* 2013). According to the conducted census, there are 631.122 farms in Serbia and total area of utilized land for agriculture is 3.355.859 ha. The total livestock number includes cattle 908.990, sheep 1.729.278 and goats 235.576 heads. Nutrition is the most important paragenetic factor, and it should follow the genetic potential of the animal. It is well known that the costs of livestock feeding in milk production can amount to 65-75% of total production costs. In the next period, because of the price of final products of animal origin, special attention should be focused on this area, which ultimately should lead to significant rationalization in Serbian livestock production, i.e. production of milk and meat. The milk production in Serbia is organized on farms with size in the range between 1.3 ha and up to 4000 ha *(Bogdanović et al., 2012)*. Development and economic efficiency of meat production largely depend on the current and potential opportunities for the development and exploitation of cheap sources of roughage. According to recent researches *(Bogdanović et al., 2013)*, about 92% of Serbian farmers prepare their own livestock feed, while 55% of farmers buy ready-made concentrates from animal feed manufacturers. Farmers that prepare their own feed, mainly produce the entire amount of forage and one part of the concentrated feed. In terms of forage, farmers prepare hay from grasses, red clover or lucerne, corn silage and, increasingly, haylage. In the mixtures for sown grassland, legume component must be included with about 30% in sward *(Stošić and Lazarević, 2007)*. Recommended species for lowland mixtures in Serbia is lucerne, in the hilly regions red clover and birdsfoot trefoil must be included, and for mountainous regions suitable legume is white clover. Important problem is unsatisfactory distribution of yield in growing season. On natural grassland first cut participate more than 90% in yield. Better distribution can be achieved with earlier cutting.

Even distribution on sown grassland can be achieved with sowing mixtures of species with different maturity period. Market provides varieties with different period of maturity and better yield distribution can be made using for earlier mixtures red fescue, for middle vegetation season cocksfoot or perennial ryegrass, and for late cutting timothy. Yield of sown grassland could reach up to 15 t ha^{-1} of dry matter (DM) *(Stošić and Lazarević, 2007)*.

There is no doubt that sown grasslands are in advantage over natural ones because they have better yield, production and quality (Lazarević et al., 2005). They are rarely formed within farm systems as an intensive type of production. It is estimated that in expanding time of sown grasslands during 80's of last century, they occupied about 150.000 ha, but today those swards are reduced significantly primarily due to a dramatic decline in livestock fund.

Technological solutions in the field of nutrition of fattening cattle and sheep depend on many factors, primarily the specific areas (plains, hills or mountains). Further development and improvement in the ruminant diet should largely be directed to the hilly area and utilization of important pasture areas (over one million ha), since they can thus be most effectively utilized. In present period those areas are mainly neglected with low biological production (1.9 t ha^{-1} DM on meadows and 0.4 t ha^{-1} on pastures) *(Lazarević et al., 2005)*.

According to some authors, lowland grasslands are of marginal importance in Serbia *(Stošić et al., 2005)*, since they are located on border soil types and have great competition in intensive field production of livestock feeds, but recent researches upbear spreading of sown grasslands in the Serbian valleys and plains *(Simić et al., 2006, 2011, 2012, Vasiljević et al., 2010, Nešić et al., 2006, Tomić Z. et al., 2012, Tomić D. et al., 2012)*. Main limitations in using of grassland resources is process of depopulation at the hilly-mountainous regions and migrating to urban centers and areas, which has been rapid during last decades. This affected the movement of livestock production in the lowlands, close to major urban centers.

It is known that mixtures of perennial grasses and legumes produce nutritionally balanced forage *(Sima et al., 2010)*, while providing a part of the protein, carbohydrate and mineral requirements. The choice of mixtures of perennial grasses and legumes, unlike pure crops of perennial grasses and legumes for the meadow establishment, provides the prerequisites of sustainable forage production *(Fornara and Tilman, 2008, Helgadóttir et al., 2008)*. Grass/legume mixtures are important for production of high quality forage, especially in conditions of lowland livestock production, in conditions of farm *housing (Tomić et al., 2011)*. Grass/legume mixtures often give the best performance. It is necessary to choose a companion grass with similar development and competitiveness. The most important perennial grasses in Serbia include Italian and

perennial ryegrass, tall oatgrass, meadow, tall and red fescue, cocksfoot, timothy and Kentucky bluegrass, while main perennial legumes are lucerne, red and white clover, birdsfoot trefoil and sainfoin *(Stanisavljević et al., 2010)*.

Cattle and sheep are in the best possible conditions to transform a rough forages low in nutritional value into highly digestible proteins with high biological value, which are important in the human diet (beef and lamb, etc.). Milk producers have to find cheaper and more competitive opportunities in nutrition, to reduce production costs, including a higher proportion of feed from meadows and pastures *(Petrović et al., 2013)*. Also, recent problems in livestock nutrition caused by aflatoxins could be controlled with nutrition mainly oriented to hay. Fodder produced from meadows could preserve the environment from the effects of livestock production. On annual basis, in Europe, it is common for 50 to 75% of cattle and 90 to 95% of sheep fodder requirements to be met by grasslands because grasses are a natural food for ruminants.

Quantitative features

A wide range of grass species used for hay production enabling grass/legume mixtures adaptation to various weather and soil conditions as well as the way of utilization. Establishment of meadow mixture with appropriate legume species is thought to be a major problem. Widely adapted forage legumes are increasingly important, but there are some constraints: lucerne, mainly used in Serbia for hay production, does not tolerate acid soils (pH below 6.2), red clover has a short life span relative to most other plant species, birdsfoot trefoil possesses very little competitive ability, while white clover has low growth habit and associated low dry matter yield. Fresh legumes may cause bloat, but it does not occur with birdsfoot trefoil. Establishment of the legumes can be critical period of growing. This failure may have occurred because of competition with weed species during first production year. Forage yield of red clover is lower in first production year, but could be increased when clover is grown in mixture with grass. Mixtures realize much greater productivity on more fertile soil, such as at lowlands. On lower altitudes the most productive are the mixtures of red clover with Italian ryegrass, and lucerne with orchardgrass *(Lazarević et al., 2005)*. Although the forage of lucerne contains more crude protein as compared with red clover, the advantage of red clover is the presence of enzyme polyphenol oxidase (PPO), which improves protein efficiency during digestion of ruminants *(Sullivan and Hatfield, 2006)* and suppresses proteolysis during forage ensiling *(Pahlow, 2003)*.

Both red clover and Italian ryegrass produce a vast amount of bulk, particularly Italian ryegrass in its first full harvest year from an autumn sowing *(Simić et al., 2011)*. Red clover is an ideal companion for Italian ryegrass. Upright, open

growth of Italian ryegrass permits the right amount of space and light for the red clover plants to establish and produce good leaf growth and develop a deep root structure. Some recent researches evaluated differences in yield, persistence and other features important with respect to agricultural production among red clover varieties from Serbia grown in simple mixtures with high productive Italian ryegrass (tab. 1, 2). Satisfactory yields of red clover crop alone or in mixture with Italian ryegrass on acid soils can be achieved if the repair is carried out by introduction of lime fertilizers. Application of liming caused a significant increase in hay yield in both cases of red clover growing (alone or in mixture). By growing of red clover in mixture with Italian ryegrass, regardless of soil liming, there was achieved a significantly higher hay yield, in relation to the pure stand of red clover *(Tomić D. et al., 2012)*.

Red clover achieved the highest yields in the first year after stand establishment *(Hejduk and Knot, 2010)*. However, sometimes depends on weather conditions, pure sown Italian ryegrass realizes the highest forage yield in autumn stand, especially with N application (tab. 1, 2). The highest yield was obtained using 100 kg ha^{-1} N, confirming that lower rates of nitrogen fertilizers affect primarily the increase of forage yield. Autumn sowing stand was more productive than spring sowing stand and reached total herbage production levels of 10.85 t DM ha^{-1} compared with 4.06 t DM ha^{-1} for ryegrass sown in spring.

Table 1. DM yield of Italian ryegrass as pure stands and mixture with red clover (kg ha^{-1}) during 2009 and 2010 growing season *(Simić et al., 2012)*

Treatment	2009			2010			2009-2010
	1st cut	2nd cut	Total DM	1st cut	2nd cut	Total DM	Cumulative DM
IR+ 0 kg ha^{-1} N	2230^{c}	212^{c}	2442^{c}	1525^{d}	383^{c}	1908^{d}	4350^{c}
IR + 100 kg ha^{-1} N	3831^{ab}	235^{c}	4066^{abc}	2743^{c}	366^{c}	3109^{c}	7175^{b}
IR + 150 kg ha^{-1} N	3122^{bc}	479^{c}	3601^{bc}	4013^{b}	478^{bc}	4491^{b}	8092^{ab}
IR + 200 kg ha^{-1} N	4389^{ab}	309^{c}	4698^{ab}	5380^{a}	898^{a}	6278^{a}	10976^{a}
IR+RC (75:25%)	3839^{ab}	1017^{b}	4856^{ab}	5043^{ab}	802^{ab}	5845^{a}	10701^{a}
IR+RC (50:50%)	4077^{ab}	1553^{a}	5630^{a}	5193^{a}	791^{ab}	5984^{a}	11614^{a}
IR+RC (25:75%)	4612^{a}	1275^{ab}	5887^{a}	5050^{ab}	1024^{a}	6074^{a}	11961^{a}
$LSD_{0.05}$	1479	386	1824	1078	395	1097	2262

*Means in columns followed by the same letter are not significantly different by Fisher's protected LSD values (P=0.05)

Different red clover varieties reached the highest yield of forage production in the ratios 50:50% with Italian ryegrass (tab. 2). Similar results were obtained by *Vasiljević et al. (2010)*. Botanical composition of autumn sowing stand showed that grass component represented larger part than it had been at establishment. Nitrogen fertilization bounded weed growth at second cutting and other herbs represented only

a small part. Spring sowing stand and its first production year gave more uniform range of herbage yields. The highest yields were obtained with larger share of red clover component. The red clover contribution to the total DM harvested of the mixtures was the largest at second cutting of spring sowing, also weed content was markedly reduced in comparison with other cuttings. DM yield and share of red clover in mixtures are higher in the second year compared with the first year of establishment of trial, probably related to the better persistence and cumulative effect of red clover than Italian ryegrass (tab. 1).

Table 2. DM yield of Italian ryegrass (IR) and red clover (RC) as pure stands and mixtures in the first production year, with two different times of stand establishment (t ha^{-1}) ***(Simić et al., 2011)***

Treatment	Autumn sowing			Spring sowing		
	1st cut	2nd cut	Total DM	1st cut	2nd cut	Total DM
IR	5.22^{b*}	1.04^{a}	6.26	2.05^{b}	0.26^{b}	2.25
IR + 100 kg ha^{-1} N	9.80^{a}	1.05^{a}	10.85	3.83^{ab}	0.23^{b}	4.06
IR + 200 kg ha^{-1} N	8.32^{ab}	1.09^{a}	9.41	4.39^{a}	0.31^{b}	4.70
IR+RC Una (75:25%)	5.91^{b}	0.76^{a}	6.67	3.84^{ab}	1.02^{a}	4.86
IR+RC Una (50:50%)	7.53^{ab}	0.71^{a}	8.24	4.08^{ab}	1.55^{a}	5.63
IR+RC Una (25:75%)	4.53^{b}	0.92^{a}	5.45	4.61^{a}	1.28^{a}	5.89
RC Una	1.36^{c}	1.24^{a}	2.60	4.15^{ab}	1.14^{a}	5.29
IR+RC K-17 (75:25%)	6.14^{b}	0.86^{a}	7.00	3.27^{ab}	1.04^{a}	4.31
IR+RC K-17 (50:50%)	8.11^{ab}	1.03^{a}	9.14	4.10^{ab}	1.33^{a}	5.43
IR+RC K-17 (25:75%)	4.71^{b}	0.79^{a}	5.50	2.93^{ab}	1.43^{a}	4.36
RC K-17	4.72^{b}	1.24^{a}	5.96	4.31^{a}	1.52^{a}	5.83

*Means in columns followed by the same letter are not significantly different by Fisher's protected LSD values (P=0.05)

Considering the obtained yields on the cumulative DM for two production years (tab. 2), a high yield was achieved in all mixtures of Italian ryegrass and red clover, which is similar to the yield of the pure sown Italian ryegrass with the highest added rate of N fertilizer (200 kg ha^{-1}). Generally, the influence of mixture ratio was less marked than sowing date. The sowing date affects total DM yield of Italian ryegrass/red clover mixture.

Land equivalent ratio (LER) index is the sum of the fractions of the intercropped yields divided by the sole-crop yields. As the amount of land needed to grow a pure stand to gain the same yield as an intercrop, LER showed that the first cutting in autumn sowing of Italian ryegrass/red clover gained the best efficiency of the production area utilization (tab. 3). A companion grass had little effect on red clover yield in the mixed swards. Its presence also reduced the ingress of unsown species. The botanical composition ratio of Italian ryegrass/red clover mixture best

persist into the first production year at the second cut of autumn sowing, but the seed rate effects were weak at other cuttings (tab. 3).

Table 3. Relative yield (RY) of dry matter and total LER index for intercropped red clover and Italian ryegrass per cutting *(Simić et al., 2011)*

Intercrop	autumn sowing				spring sowing			
	1st cut		2nd cut		1st cut		2nd cut	
	RY	LER	RY	LER	RY	LER	RY	LER
IR:RC Una (75%:25%)	1.13:4.35	5.48	0.73:0.61	1.34	1.87:0.93	2.80	4.80:0.73	5.52
IR:RC Una (50%:50%)	1.44:5.54	6.98	0.68:0.57	1.25	1.99:0.98	2.97	7.33:1.11	8.44
IR: RC Una (25%:75%)	0.87:3.33	4.20	0.88:0.74	1.62	2.25:1.11	3.36	6.01:0.91	6.93
IR:RC K-17 (75%:25%)	1.18:1.30	2.48	0.83:0.69	1.52	1.59:0.76	2.35	4.88:0.68	5.56
IR:RC K-17 (50%:50%)	1.55:1.72	3.27	0.99:0.83	1.82	2.00:0.95	2.95	6.27:0.88	7.14
IR:RC K-17 (25%:75%)	0.90:1.00	1.90	0.76:0.64	1.40	1.43:0.63	2.05	6.74:0.94	7.68

There is a problem of legume maintenance in meadow mixtures, what affects adversely the reduction of herbage quality. Growing of lucerne in mixtures with grasses has numerous advantages, such as higher yields than pure stands during vegetation, better quality of forage, as well as more economically efficient production due to reduced use of nitrogen fertilizers *(Tomić et al., 2011, Tomić Z. et al., 2012)*. Fertilization from 0 to 100 kg N ha^{-1} causes a significant increase of yield of pure crops and grass/legume mixtures, and difference may reach in average of 1.43 t ha^{-1} *(Tomić Z. et al., 2012)*. Cocksfoot has proven to be more persistent and yielding grass for cultivation in mixture with lucerne compared to tall fescue (*Nešić et al*., 2008c). This indicates great competitive ability of cocksfoot in relation to tall fescue and lower suitability of their combination in mixture with lucerne.

Grass mixture composed of meadow grasses (same percentage of cocksfoot, timothy and meadow fescue - 25%, tall and red fescue - 10% and 5% Kentucky bluegrass) may produce notable DM yield in Serbian lowlands during 2 production years (tab. 4), and highest yield could be obtained with spring nitrogen application of 100 kg ha^{1}N.

Table 4. DM yield of meadow grass mixture (first cut) in 2004-2005 year (t ha^{-1}) *(Simić et al., 2006)*

	0 kg ha^{1}N	50 kg ha^{1}N	100 kg ha^{1}N
2004	1.61	2.36	3.06
2005	1.92	4.06	5.41
Total	3.53	6.42	8.47

Fertilization

Nitrogen acquisition is one of the most important factors for plant production, and N contribution from biological N_2 fixation can reduce the need for industrial N fertilizers *(Carlsson and Huss-Danell, 2003)*. When grown in mixtures with grasses, red clover took a large fraction of their nitrogen from N_2 fixation (average around 80%), regardless of management, dry matter yield and location. *Wacquant et al. (1989)* suggest that NO^{3-} enrichment in the soil results from N excretion from active nodulated roots of the legume, accounting for the increase in both biomass and nitrogen content of the companion grass in grass-legume association. N fertilization improves competitive abilities of grasses and increases productivity *(Nešić et al., 2008b,c, Tomić et al., 2011, Simić et al., 2006, Vasiljević et al., 2010)*. Introduction of high rates of N fertilizer can also lead to accumulation of NO^{3-} in plants, but also in soil, which can have negative consequences *(Nesic et al., 2008a)*. With a view to producing higher yields, large quantities of N are often used. However, such quantities can cause accumulation of NO_3^- in forage which is potentially harmful for livestock health. Therefore, it is necessary to reduce N fertilization to the detriment of yield but in favour of quality.

High annual DM yield of red clover in mixture with Italian ryegrass, with high nutritive value and without the use of nitrogen fertilizer have been reported *(Simić et al., 2011, 2012, Vasiljević et al., 2010)*. This in turn means the red clover can very efficiently fix atmospheric nitrogen via its root nodules and therefore minimise the requirement of adding artificial N to the crop. Any grass seed ley containing red clover also has a much higher protein content (compared to ryegrass alone) maximising voluntary intake of the available forage. White clover and lucerne, in mixtures with grass, also appear to be economically more attractive than pure grasses produced on swards receiving 400 kg of N per hectare *(Wilkins and Paul, 2001)*. Levels of N leaching from fields with grass/legume mixtures is reduced. Recent researches support using of zeolites as a binding agent for ammonia ions and as a soil additive on grasslands *(Simić et al., 2013)*. Application of the organic fertilizer enriched with zeolite on grasslands can be beneficial in achieving and maintaining a high output of herbage.

Composition

It is necessary to consider the choice of species included in sown meadow mixtures, i.e. their competitive traits. Compatibility of grasses and legumes depends on morphological and physiological characteristics of species as well as characteristics of given association, its interaction with applied practices, climatic, edaphic and biological conditions in which an association is developing *(Nešić et al.**,** 2008c)*. Also, instability of simple grass/legume mixtures with only one grass and one legume species could be a major problem. High amount of nitrogen may have a depressive effect on proportion of lucerne although it enlarged the proportion of grass in the mixture *(Nešić et al., 2006)*. Share of weed species decreased in the second production year, especially ratio of weed was lower in mixture compared to pure sown lucerne.

Recent researches in Serbia included intercrops with different red clover varieties (K-17 and Una) and tetraploid Italian ryegrass in different proportional ratios (100:0%, 75:25%, 50:50%, 25:75% and 0:100%), were trying to find best ratio for Serbian conditions (fig. 1, 2, 3). Italian ryegrass sown alone was top-dressed with nitrogen rates of 100 and 200 kg ha^{-1}. Also, potential of ryegrass/clover mixture to achieve and maintain a high output of herbage in the first production year, with different time of stand establishment was explored. Red clover is probably best grown in association with grass as the mixture increases dry matter yields and reduces sward weed problems. When seeded with legumes, Italian ryegrass provides early protection of the soil, suppresses weeds, and acts as a nurse crop. Italian ryegrass contributed less to the total yield in the mixtures, because it had lower ratios in the structure of swards than those used at sowing (fig. 1, 2, 3). A Italian ryegrass/red clover sward may have advantages over a pure Italian ryegrass sward fertilized by high rates of N, providing high yield with more sustainable land use system. Persistency can be a problem for Italian ryegrass and red clover.

Yields and average ryegrass contents declined in the second cut, particularly in second production year or in dry summer conditions. Italian ryegrass had lower ratios in the structure of swards than those used at sowing, both at the first and the second cut. Red clover was absolutely dominant in the second cuts of both years and this can be explained by the dry weather during summer growth, conditions leading to poor regrowth of ryegrass and more favourable for clover. Generally, unsown species contributed progressively more to the total yield of monocultures, than to the mixtures, where the invasion of unsown species was minimal. Weeds had ratios of 4-35% in the botanical composition of all treatmants at the first cut, while at the second cut their ratios decreased by less than 10% in the majority of variants. The ratios of weeds decreased in the botanical composition of

swards of all mixtures by the second cut, which is in agreement with those reported by *De Vliegher and Carlier (2008)*.

The most persistent red clover variety should be used preferably for lays and for the establishment of permanent grasslands or sod seeding. The use of more persistent variety would lead to reduced requirements of nitrogen fertilizers, increased forage yield and quality as well as to extended intervals between red clover seeding into sod. This would consequently lead to decreased inputs in cattle rearing and reduced costs of animal products. It is necessary to test persistence under local conditions together with companion grass.

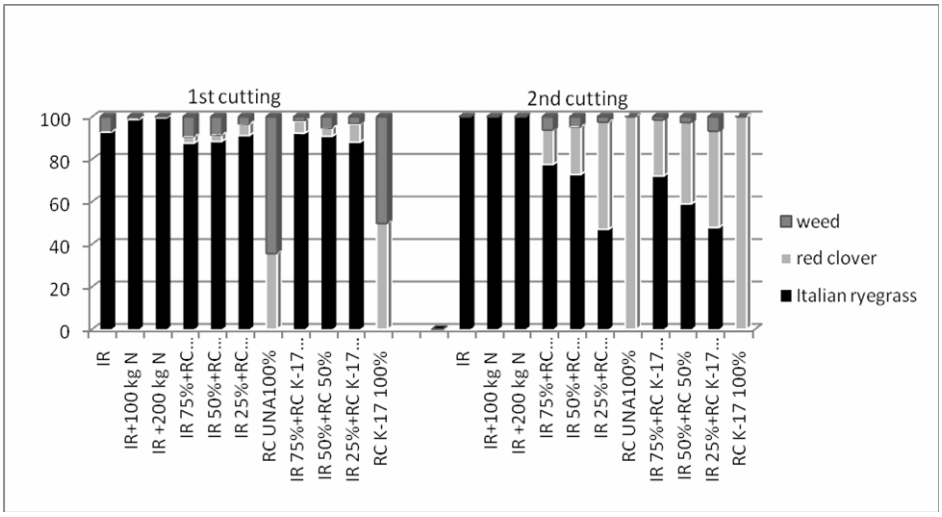

Figure 1. Share of each component (botanical composition) in mixtures per cutting (%), autumn sowing *(Simić et al., 2011)*

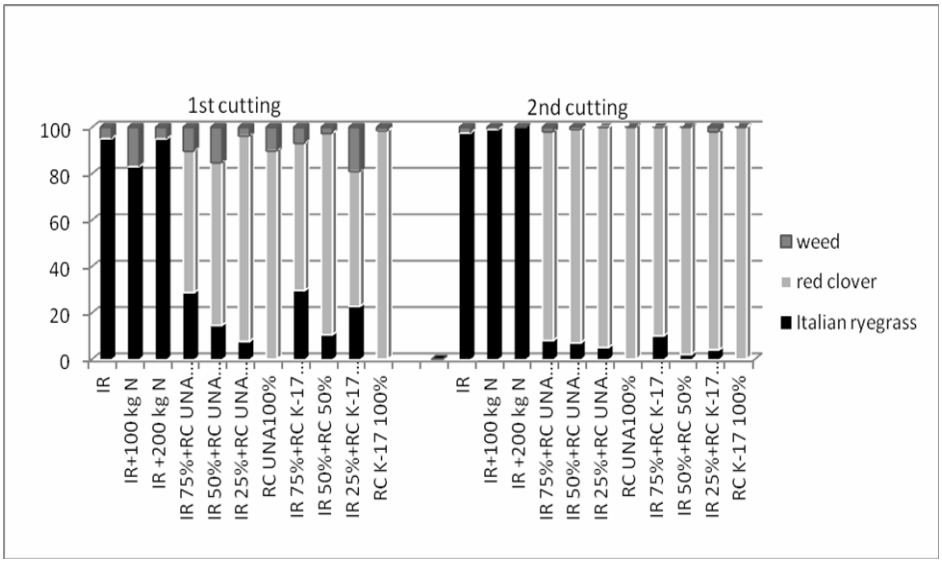

Figure 2. Share of each component (botanical composition) in mixtures per cutting (%), spring sowing *(Simić et al., 2011)*

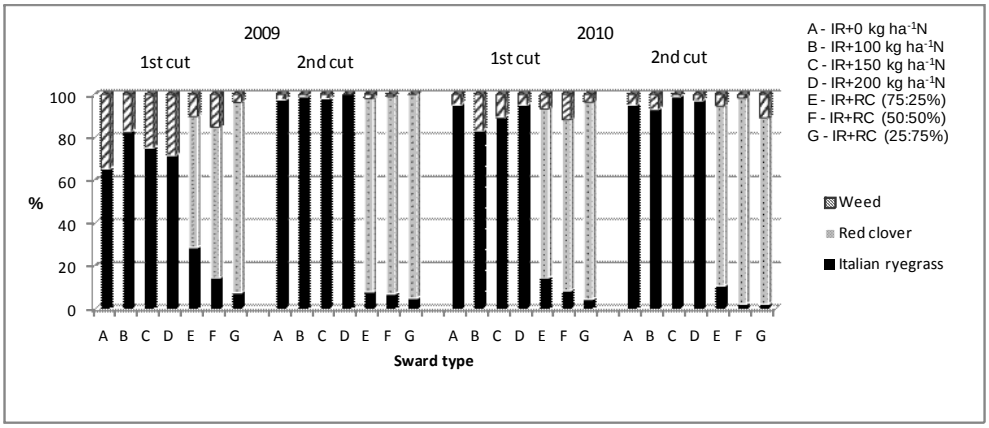

Figure 3. Botanical composition of sward (%) with pure sown Italian ryegrass (IR) and mixture with red clover (RC) *(Simić et al., 2012)*

Application of liming caused a significant positive effect on proportion of red clover, grown alone or in mixture with Italian ryegrass *(Tomić D. et al., 2012)*.

Hay quality

A legume/grass sward may have advantages over a pure legume sward nutritionally. Conservation of forage in the form of hay can cause a decrease of crude protein (CP) content, because of improper handling, excessive drying or loss of leaves. Legumes are characterized by significantly higher CP content compared to grasses. Content of CP varied significantly in relation to crop and fertilization. Recent researches in Serbia compared forage quality of different pure sown species (lucerne, cocksfoot, meadow fescue and perennial ryegrass) with their mixtures (tab. 5). The highest content of crude protein (CP) in pure stand is obtained in lucerne (14.9%), while in mixtures lucerne and cocksfoot provided the highest percentage of CP (12.68%). Nitrogen fertilization emphasizes the increase of CP from 9.90 to 13.10%.

Table 5. CP, CF, NDF and ADF in pure crops and mixtures depending on fertilization (%) ***(Tomić Z. et al., 2012)***

Main effect	CP	CF	NDF	ADF
Crop				
lucerne	14.29	27.81	44.48	36.34
cocksfoot	11.10	28.78	63.59	37.44
meadow fescue	9.23	27.41	61.68	37.55
perennial ryegrass	8.94	26.58	52.69	32.57
lucerne+cocksfoot 50:50	12.68	28.76	59.22	37.34
lucerne+cocksfoot+meadow fescue 50:25:25	13.08	29.00	61.30	36.31
lucerne+cocksfoot+meadow fescue 25:50:25	11.33	27.65	63.56	38.04
lucerne+cocksf.+mead.fescue+perennial ryegrass 40:20:20:20	12.29	29.28	60.95	39.19
LSD 0.05	1.24	ns	2.47	2.50
N fertilization (kg ha^{-1})				
0	9.90	28.11	57.61	36.84
50	11.86	28.45	58.57	37.08
100	13.10	27.91	59.11	36.62
LSD 0.05	1.07	ns	ns	ns

ns – not significant

Fertilization may increase significantly the yield of CP by 194.1 kg ha^{-1} in monocultures and by 323.2 kg ha^{-1} in grass/legume mixtures *(Tomić et al., 2011)*. The highest CP yield was obtained from perennial ryegrass (495.5 kg ha^{-1}), while four-components mixture (lucerne+cocksfoot+meadow fescue+perennial ryegrass) gained 568.0 kg ha^{-1}. N fertilization significantly increased CP yield of monocultures, as well as their mixtures.

According to *Simić et al. (2006, 2007, 2009)*, nitrogen application could change forage quality parameters of meadow grasses and grass mixtures, but final

hay energy value is nearly equal with and without N-spring application by years (tab. 6). Net energy value of meadow grass mixture is properly changed by N fertilizing in the second production year, and NEL and NEM are decreased by increasing rates of N (NEL 4,23-4,10 MJ kg^{-1}, i.e. NEM 4,03-3,86 MJ kg^{-1}), while in the first production year failed to exert influence of N. However, N spring application increases hay yield and influences energy value of hay by quantitative features.

Table 6. Forage quality parameters and energy value of meadow grass mixture (first cut) ***(Simić et al. 2006)***

Parameter	year	0 kg ha^{-1} N	50 kg ha^{-1} N	100 kg ha^{-1} N	$LSD_{0.05}$
crude protein	2004	126.4*	144.0	145.2	12.8
	2005	86.8*	104.7	106.2	12.6
crude fibre	2004	267.6	270.0	281.8	14.9
	2005	247.2*	302.3	320.6	20.8
crude fat	2004	28.4	32.3	25.6	8.4
	2005	28.2	33.7	22.2	9.2
NFE	2004	386.7*	343.1	341.9	25.2
	2005	459.6*	376.6	363.1	22.5
NEL MJ=	2004	4.07	4.07	4.06	0.110
	2005	4.23	4.19	4.10*	0.076
NEM MJ=	2004	3.84	3.83	3.81	0.110
	2005	4.03	3.96	3.86*	0.087

* significant at the level of ($p<0.05$)

Conclusion

It could be concluded that high forage yield from sown meadows could be obtained using suitable legume component in mixture with grasses. Based on the results obtained from researches in Serbia, Italian ryegrass can be a suitable companion grass for red clover in the first production year. Its superior yielding capacity over other grasses under a conservation management can be coupled to advantage with red clover to give a sward which is essentially stable, at least over a 2- to 3-year cropping period, although giving slightly reduced yields in the first production year in Serbian conditions. Better stands of Italian ryegrass/red clover mixture were obtained from sowings made in autumn. There was a significant interaction between seed rate and date of sowing. A mixture ratio of 50:50% proved the most satisfactory. The practical agricultural implications of using Italian ryegrass/red clover mixtures is production of high dry matter yield of good quality herbage with reduced requirements for nitrogen fertilizers.

Growing of lucerne and grasses in mixtures in Serbian lowlands has numerous advantages, such as higher yields than pure stands during vegetation, better quality of forage, as well as more economically efficient production due to reduced use of nitrogen fertilizers. Utilisation of grass/legume meadow mixtures in Serbian lowlands instead of grass or legume monocultures is a sensible alternative for low input and efficient agricultural systems that reduce production costs and promote alternative, sustainable forage production.

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Livadske smeše u Srbiji: izazovi i vidici

A. Simić, Z. Tomić, S. Vučković, Z. Bijelić, V. Mandić

Rezime

U radu su objedinjeni novija iskustva i rezultati u proizvodnji stočne hrane sa sejanih livada u Srbiji, sa posebnim naglaskom na značaj očuvanja leguminoznih vrsta u smešama i sa tim povezanom agrotehnikom. Takođe, ispitivanja procenjuju dobiti od travno-leguminoznih smeša u smislu proizvodnje krme, ispitujući da li kombinacije različitih vrsta unapređuju stabilnost smeša i ispituju modele odgovora na većem broju primera u različitim uslovima gajenja.

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GRASS-LEGUME SILAGES

Z. Bijelić[1], Z. Tomić[1], D. Ružić-Muslić[1], V. Mandić[1], A. Simić[2], G. Marinkov[1], Lj. Stojanović[1]

[1]Institute for Animal Husbandry, 11080, Belgrade-Zemun, Republic of Serbia
[2] Faculty of Agriculture, University of Belgrade, Zemun-Belgrade, Republic of Serbia
Corresponding author: zonesh@gmail.com
Invited paper

Abstract: The aim of this paper is to present the possibilities of preparing silage from grass-legume mixtures according to their fermentable characteristics, factors affecting silage quality, directly or indirectly, with special reference to the impact of belonging to the botanical species and crop management, nitrogen fertilization and stage of maturity of the crop at the time of exploitation. Plant species used for the preparation of feed mixtures are characterized by different chemical and fermentable characteristics. Therefore, to get the silage which satisfies specified requirements for nutrients, great attention must be paid to the proper selection of species for the preparation of mixtures. In addition to the structure of the mixture, the fermentation quality and chemical characteristics of silage are influenced by growing practices applied during the growing season. Fertilizer, although it has a favourable effect on seed yield, has quite a negative effect on fermentation and silage quality because it increases the buffer capacity and reduces the content of fermentable sugars. Plants at different growth stages, are characterized by different chemical characteristics and fermentable capabilities. Crop utilization in optimal stage of development can provide high quality product.

Key words: grass-legume silages, N fertilization, stage of maturity

Introduction

Modern and intensive production of milk and meat cannot be imagined without the use of silage. Silage is feed of specific taste and smell, which aims to preserve the nutritional value of green plants in the long run. The term silage is a complex process consisting of a series of activities aimed at obtaining high-value preserved feed. Silage quality means high content in certain nutrients and their lowest possible microbial decomposition. In order to ensure the best quality silage, plant material of high quality is required, controlled conditions during the preparation and proper conservation. In addition, the quality of the silage can be affected by many other factors that can be divided into climatic and agro-technical

factors. Climatic conditions in the field, high temperature, drought or rain, and climatic conditions during storage, can significantly affect the quality of the silage. The agro-technical factors are following: botanical composition, level of fertilization (especially N), the frequency of cutting, phenological stage of cutting, wilting or dry matter content of the silage mass, the addition of chemical and microbiological additives, etc.

Legumes are characterized by high nutritional value, long period of exploitation, resistance to drought and low temperatures and the possibility of different modes of use. They are rarely used in the green state, due to possible health problems that they may cause in ruminants. The most common method of their conservation is drying, but it causes significant loss of nutrients. However, ensiling can preserve nutrients and get forage nutritive value, which is very similar to green forage. But with this method of preservation there are some problems, such as lack of sufficient quantities of sugar that are necessary for the progress of fermentation and to obtain good quality silage *(Dinić et al., 1988*). This problem can be overcome by certain procedures, such as the cultivation of legumes with grasses as grasses provide the necessary amount of sugar for ensiling.

Plant species used for the preparation of feed mixtures are characterized by different chemical characteristics, including different ability for ensiling. In this case, serious attention must be given to species selection in the preparation of mixture, which will be used for silage.

Cultural practices applied during the growing season can have a significant impact on the course of fermentable process and silage quality. Fertilizer, although it has a favourable effect on plant yield has quite a negative impact on the quality of silage fermentation and silage material and it increases the buffer capacity and total nitrogen content and decreases the content of fermentable sugars and dry matter. Also, the application of N fertilizer can stimulate the growth of harmful microorganisms and mycotoxin production in silage *(Lemmens et al., 2004)*.

Plants at different growth stages are characterized by different chemical characteristics and fermentable capabilities. Crop utilization in optimal stage of development can provide high quality product. However, the orientation of production and botanical origin of plants which determine the stage of development in which the crop will be used. Perennial legumes should be ensiled in the budding stage and early flowering, and grass at stage of ear formation *(Djordjevic and Dinić, 2003)*.

The aim of this paper is to show the possibility of preparing silage from grass-legume mixtures according to their fermentable characteristics, factors affecting silage quality, directly or indirectly, with special reference to the impact of belonging to the botanical species and crop management, nitrogen fertilization and stage of maturity of the crop at the time of exploitation.

Importance of grass-legume mixtures

Improvement of livestock production and strengthening of its economic importance is in direct correlation with the production of quality and cheap fodder. In Serbia, the statistics from the year 2012 show 590.927 hectares under the meadows and 702.887 hectares of pastures *(RZS, 2012)*. Comparing statistics from previous years, we can conclude that the production area has decreased. For these reasons, attention should be directed towards planted grasslands, or mixtures of grasses and legumes. There are numerous benefits of grass-legume mixtures that lead to their cultivation. One of them is nitrogen fixation. The effect of nitrogen fixation increases in grass-leguminous mixtures, hence meeting to a large extent the needs of legume and grass for N and thus reducing the need for N fertilizers. Legumes in the mixture meet 92-95% of their N requirements *(Brophy et al., 1987),* while 29-36% of the total fixed nitrogen is taken by the grasses *(Heichel and Henjum, 1991)*. The mixtures are characterized by high production and even influx of forage during the entire growing season

Table 1. Yield of dry matter of lucerne mixtures per cuts (t ha^{-1}) (source *Nešić, 2006*)

Mixtures	Cut				Total yield
	I	II	III	IV	
Lucerne	5.46	5.39	3.37	1.68	15.91
Lucerne + cocksfoot	5.22	4.58	2.50	1.58	13.83
Lucerne + cocksfoot + tall fescue	5.26	5.88	2.41	1.60	15.47
Lucerne + cocksfoot + tall fescue + sainfoin	6.37	5.15	1.85	1.49	14.88

In addition to increasing durability and grassland productivity, mixtures provide a higher concentration of protein, digestibility, nutritional value, reduce the concentration of cellulose and encourage mineral balance of forage crop compared to pure grass (*table 2*). Cattle can be fed the green feed because the possibility of bloat is reduced, and is characterized by better performance compared to cattle fed pure crops *(Eerens et al., 1997; Sanderson et al., 1999)*.

Table 2. Average quality values, ADF, NDF, IVTD, and CP for monocultures and mixture in Norway, *(source Sturludóttir, 2011)*

Grassland type	ADF (g kg^{-1})			NDF (g kg^{-1})			IVTD (g kg^{-1})			CP (g kg^{-1})		
	1	2	3	1	2	3	1	2	3	1	2	3
Timothy	303	298	316	584	585	581	803	837	825	62	73	78
R. clover	274	260	259	410	420	418	831	851	862	146	107	112
Mixture	307	295	312	541	531	555	815	838	827	98	104	98

ADF-Acid Detergent Fiber; NDF- Neutral Detergent Fiber; IVTD-in vitro dry matter digestibility; CP- crude protein

Legumes are very difficult ensilaged because of the lack of sugar minimum necessary for good silage preparation. For this reason, their joint cultivation with grasses allows this type of conservation, as grasses provide sugars that stimulate fermentation, resulting in highly valuable animal feed, which is suitable for feeding all species of domestic animals *(Djordjevic and Dinić, 2003)*. The study of fermentable characteristics of lucerne and smooth bromegrass, *Wang et al. (2012a),* showed that the content of water soluble carbohydrates in fresh lucerne was 32.6 ± 1.13, and in fresh forage of smooth bromegrass 54.6 ± 0.92 g kg^{-1} DM. In addition *Han et al. (2006)* examined the characteristics of lucerne silage, English ryegrass, and their mixtures, and concluded that the content of easily soluble sugar in ryegrass was 139.0 g kg^{-1} DM and to be significantly different from the content in pure lucerne crop of 77.0 g kg^{-1} DM, and in the mixture of lucerne and English ryegrass of 79.0 g kg^{-1} DM in the first cut and in the second of 100.0 g kg^{-1} DM compared to lucerne of 71.0 and mixture of 83.0 g kg^{-1} DM.

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Factors influencing the silage quality

Ensiling green fodder is the traditional method of preserving animal feed which is important in the production of hay or direct feeding using green mass. The technology of preparation is simple and involves the compression of feed to produce a totally anaerobic environment *(Danner et al., 2003)*.

In conditions of very pronounced deficits and high energy prices *Djordjevic et al. (2000)* argue that the silage is the most effective solution in order to get cheap and high quality feed. However, successful ensiling can be difficult due to numerous problems such as a high proportion of moisture, high buffer capacity of forage, insufficient amount of fermentable carbohydrates, soil contamination, etc. On the other hand, a small share of protein and low digestibility are the main reasons why some ensiled feeds have low nutritional value and are not used to a greater degree in ruminant nutrition.

The most important factors that should be respected prior to the preparation of silage is to ensure adequate dry matter content in the silo material as well as the right amount of sugar and buffer capacity (*Knický, 2005).*

In order to make good quality silage it is necessary to produce silage from wilted materials containing DM from 320-380 g kg^{-1}. Slightly lower values are recommended for sown grasslands, and slightly higher for the natural.

The required amounts of dry matter for fermentation are provided by wilting biomass after cutting. Wilting duration may vary depending on the characteristics of climatic areas. In our weather conditions, wilting is usually 12 - 24 hours *(Djordjevic and Dinić, 2003)*. By studying the impact of wilting on the quality of silage of red and white clover, *Dinić et al. (1994)* have demonstrated that

wilting leads to an increase in the pH value of 0.1, acetic acid 0.37 g kg^{-1}, lactic acid by 4.7 g kg^{-1}, and decrease of butyric acid by 0.34 g $kg^{-1}$and NH_3-N by 0.94%. In addition to drying, the dry matter content in the silo mass of grass-legume mixtures can be increased by adding the drier feed (corn stalk, straw, hay).

However, as it is not good to have the mass with a high moisture content, it is also not good to have the mass that is too dried. In excessively wet silage clostridia proliferate intensively, which can result in increased protein degradation, loss of dry matter and production of toxins. Also large amounts of silage effluent are produced that may pose a threat to the environment. On the other hand drier biomass is poorly compacted and residual air creates a suitable environment for the growth of harmful microorganisms such as toxigenic fungi *(Kung, 2001)*, so that the silage after fermentation is poor nutritional value with possible presence of significant concentrations of mycotoxins.

Sugars are the main source of fermentable compounds in the preparation of silage and together with buffering capacity affect the process of preparing silage. Therefore, they are expected to be useful indicators of crop conservation opportunities. These are mainly glucose, fructose, sucrose and fructan. Glucose and fructose are the main easy hydrolyzing sugars, and provide a substrate for lactic acid bacteria in the early stages of the silage process. In the later stages, these bacteria use disaccharides and polysaccharides in their diet. The concentration of these sugars mainly depends on the plant species and the utilization phase. In grasses that grow in the temperate zone of *Lolium* sp. have a high value of fermentable sugars in the average 175 g kg^{-1} DM, then *Phleum* sp. containing about 110 g kg^{-1} DM and *Dactylis glomerata*, which contains only 79 g kg^{-1} DM. The sugar content in the grasses increases as the share of stem increases or stem to leaf ratio. On the other hand legumes contain low concentrations of sugar. The main fermentable sugars in legumes are glucose, fructose, sucrose *(Knický, 2005)*.

These are the three most important characteristics of the silo material directly influencing the fermentation process. Besides these there are many other factors such as botanical origin, stage of development, the cycle of vegetation, cultural practices, conditions of ensiling, characteristics of the silo facility and technique of ensiling *(Đorđević and Dinić, 2003; Wyss, 2004)*.

Silage quality parameters

The quality parameters of silage include: dry matter content, pH value, content of lactic, acetic, propionic and butyric acids, as well as the content of ammonia and soluble nitrogen.

The degree of acidity or pH is one of the first, and sometimes the best criteria for assessing the quality of silage. pH indicates the type and level of

fermentation *(Amigot et al., 2006)*. Silage prepared from different plant species is characterized by different pH values. Crops with higher sugar content have the ability to reduce silage pH to the optimum level, while in crops with lower sugar content, pH remains at a higher level. *McKersie (1985)* has concluded that the optimum pH for legume silage is 4.5. According to *Loučka et al. (1997)* good silage made from grasses and clover had a pH of 3.75 and 4.23 and 5.06 of lucerne. In studies by *Dinić et al. (2000),* the pH of silage mixture of red clover and Italian ryegrass ranged from 3.63, in 50:50 ratio of species compared to value of pH of 4.00 at ratio 75:25.

Ammonia nitrogen and soluble nitrogen reflect the level of proteolytic activity and protein degradation in silage. This process is harmful because it reduces the nutritional value of the silage. The acceptable amount of ammonia nitrogen in grass and legume silages is 10% or 100 g kg^{-1} ΣN *(Djordjevic and Dinić, 2003).*

Lactic acid is a product of lactic acid bacteria activity and proper preservative, which decreases pH values of silage and inhibits the growth of harmful microbes. Substrate for its establishment is carbohydrates. As the crops differ in the content of fermentable sugars, so they differ in their ability to form lactic acid. Leguminous plants have a low capacity for which they are added during bacterial silage inoculants and substrates with high sugar content. In a well-fermented silage, lactic acid content ranges from 4.05-5.74% in grass silage from 6.60-9.72%, in clover silage *(Florek et al., 2004)*, and from 2.7-3.4% in grass-leguminous silage *(Bijelić, 2009a)*.

In addition to lactic acid, during fermentation also acetic acid occurs mainly in the early stages while still an aerobic environment. Excessive amounts of acetic acid are a sign that the aerobic conditions lasted too long and that the mass is not compressed enough. Despite the negative signs lately acetic acid obtains a positive character because it is considered a requirement for preserving the aerobic stability of silage after opening *(Danner et al., 2003)*. If the content of acetic acid is up to 5.5% of dry matter, it is considered to be good quality silage *(Djordjevic and Dinić, 2003).*

Butyric acid is a side product of fermentation and is an indicator of the presence of clostridia in silage *(McDonald et al., 1991)*. In the research of *Čunderlíková et al. (2003),* the content of butyric acid in silage mixtures, which are rated as good, varied from 0-0.12 g kg^{-1} DM

In addition to the quality of silage measured by chemical fermentative characteristics, it is important to determine the quality and the presence of harmful micro-organisms (moulds or fungi, yeasts, clostridia). They develop in the part of silage where oxygen is present. Fungus that usually occur belong to the genus *Penicillium* spp., *Fusarium* spp., *Aspergillus* spp., *Mucor* spp., *Trichoderma* spp., *Byssochlamys* spp. The most important factors that influence the growth of fungi

during fermentation and silage after opening are temperature, atmospheric composition, moisture content, pH, chemical composition and biotic factors (presence of insects). Fungus resistance to organic acids varies according to the genus and species. Lactic acid doesn´t have fungicide effect, but has a bactericidal effect unlike propionic acid, which is a potential inhibitor of fungi *(Nedělník et al., 2006)*.

Mycotoxins are secondary metabolites of fungi. If the silage is well prepared it should not be expected to contain mycotoxins, zearalenone, however, field mycotoxins, can survive in silage. It is produced by toxigenic fungi of the genus *Fusarium* spp. mainly accumulated at the end of the growing season. The highest concentration was found in leaves of plants in the plant basis. Also, fungi of the genus *Fusarium* spp. produce fumonisins and deoxynivalenol.

The effect of mixture type on quality of grass-legume silages

Botanical composition of grassland is one of the important factors affecting the fermentation since each species and each variety have different silage characteristics. *Wiessbach et al. (1974)* have reported that fermentable abilities of certain species are determined of easily soluble sugar content and dry matter content and buffering capacity. Therefore, to get the silage which satisfies specified requirements for nutrients, great attention must be paid to the proper selection of species for the preparation of mixtures. According *Weisbjer et al. (2012)* grasses are easier ensilaged than legumes because they have a higher content of water soluble carbohydrates, lower buffer capacity and lower protein content. There are also differences between different grasses and legumes, as well as within the same species, between different varieties.

Table 3. The effects of structure of mixtures on chemical and biochemical characteristics of grass-legume silage *(source Bijelić, 2009a)*

	Lucerne + cocksfoot	Lucerne + cocksfoot + tall fescue	Lucerne + cocksfoot + tall fescue + sainfoin	Lucerne	Level of significance
Dry matter (g kg^{-1})	438.1	429.9	397.0	409.4	**
Crude proteins (g kg^{-1} DM)	159.5	157.6	154.9	162.6	ns
NH_3N in ΣN	107.7	108.3	112.0	108.3	**
Soluble N in ΣN	490.4	444.8	460.3	527.1	**
Lactic acid	31.4	27.6	26.9	33.6	**
Acetic acid	13.9	13.1	15.0	12.0	**
Butyric acid g kg^{-1} DM	0.02	0.00	0.06	0.01	**
pH	4.60	4.70	4.70	4.70	ns

ns – not significant
** significant at the level of ($p<0.01$)

In the research of *Bijelić (2009a)* it has been observed that different types of mixtures as a study factor had high significant impact on almost all chemical and biochemical indicators of quality silage. The content of ammonia nitrogen in regards to total nitrogen azot was relatively low in all mixtures that can be explained by the high dry matter content, low content of butyric acid, which is also a sign of poor clostridial activity, resulting in proteolysis and creating of NH_3-N. In this example, effects of individual species on the content of ammonia nitrogen are not clearly visible, but the individual authors suggest that the grass silages have significantly lower content of ammonia nitrogen (11.0-12.7 g kg^{-1} ΣN) than legume silage from 93.3-109.4 g kg^{-1} ΣN which is typical for legumes due to higher crude protein content *(Fredeen et al., 1991)*. Moreover, among the different types of grass silages, the significant variability of ammonia nitrogen is noted, especially when it comes to cocksfoot and timothy grass silages *(King et al., 2012)*.

The content of lactic acid was positively correlated with the content of fermentable sugars. Therefore silage mixture mainly composed of grasses that contain more fermentable sugars will have more lactic acid. The content of lactic acid in the research of *Bijelić (2009a)* was highly dependent on the plant species and the structure of the mixture. There are also numerous studies that have shown a high dependence of lactic acid on the composition of the mixture *(Heikkila et al., 1992; Olt et al., 2005)*.

Share of butyric acid in research by *Bijelić (2009a)* was highly dependent on the type of mixture (Table 3). For silage mixture, increase of the dry matter content and easily fermentable sugars means a reduction of NH_3-N and butyric acid *(Kasmaei et al., 2012)*. This is confirmed by the study of *Wang et al., (2012b),* who have examined lucerne and corn silage in various proportions and concluded that with the increase in the share of corn in silage the content of butyric acid is reduced. In pure corn silage it was not present unlike the pure lucerne silage with the highest content of butyric acid.

Moulds that grow in silage can come from the field or from warehouse. They impair the quality of silage and especially develop in silages in which good anaerobic conditions were not achieved. The study by *Bijelić et al. (2009b)* demonstrated that differences in the mixture have a significant impact on the occurrence of fungi. Most present were field fungi - *Fusarium* species (80-90%), while the rest belonged to field and storage fungi (*Aspergillus* spp., *Rhizopus* spp., *Paecylomices* spp., *Penicillium* spp., *Acremonium* spp., *Alternaria* spp., *Mucor* spp). Fungi produce mycotoxins. The most common mycotoxin producers in Serbia are fungi of the genus *Fusarium* spp. *(Krnjaja et al., 2004)*. Differences in the composition of the mixture have a significant effect on the concentration of mycotoxins in silage *(Bijelić, 2009a)*, while other soil management and climatic factors can also have a significant impact. Thus, the *Codex Alimentarius*

Commission (2003) provides some measures that can prevent the production of field mycotoxins, such as crop rotation, removal of crop residues, planting resistant varieties, fertilization according to plant needs and the application of good agricultural practices to avoid plant stress from high temperatures or drought, the use of appropriate phyto-sanitary measures on seeds or crops, to minimize mechanical damage.

The effect of N fertilization on the quality of grass-legume silage

As an important agro-technical measure, which has a positive effect on increasing the production characteristics of plants, N fertilization can have a negative impact on their fermentation ability and silage quality. The negative impact of N fertilization is reflected in an increase in buffer capacity, total nitrogen content and the reduction of fermentable sugars (*Tommila et al., 1996*).

Keady et al. (2000) have investigated the effect of adding different amounts of nitrate fertilizer crop (72, 96, 120, 144, 168 kg ha^{-1}) on the quality of the silo mass and nutritive value of grass silage. As the level of nitrogen fertilization increased from 72-168 kg ha^{-1}, forage yield, the concentration of nitrate nitrogen, crude protein, ash, NDF and ADF significantly increased, while the concentration of dry matter and fermentable sugars significantly decreased. The level of fertilization had no effect on forage pH, buffer capacity and concentration of cellulose, hemicellulose and ADL. In silage, increased N fertilization decreased the dry matter content, fermentable sugars, metabolic energy, and increased content of crude protein, ADF and ADL, and had no effect on pH, concentration of ammonia nitrogen, lactic acid, acetic and butyric acids, ethanol, NDF and ash.

Fertilization with large quantities of N results in the accumulation of nitrate in forage grass *(Nešić et al., 2008a, 2008b)*, thereby increasing their concentration in the silage. The content above 1.7 g kg^{-1} DM in the silage is considered as potentially dangerous, while for fresh fodder that value is slightly higher 3.4 g kg^{-1} DM *(Tremblay et al., 2005)*.

In the three-year research of *Bijelić, (2009a)*, N fertilization had a highly significant effect on lactic, acetic and butyric acids, ammonia and soluble nitrogen and total fungi in silage, but not on pH, mycotoxins and the content of dry matter and crude protein in the first and last year of study.

The application of nitrogen fertilizer favours the development of harmful microorganisms on crop and production of mycotoxins. This was confirmed by *Lemmens et al. (2004)* in the examination of the impact of N fertilization on development of fusarium species and mycotoxin deoxynivalenol.

Table 4. Effect of N fertilization on the parameters of quality grass-legume silage *(Bijelić, 2009a)*

Treatments	kgN ha^{-1}			
	0	7	14	210
Dry matter (g kg^{-1})	352.9	334.7	341.5	343.8
Crude proteins (g kg^{-1} DM)	145.5	150.6	150.4	151.9
NH_3N in ΣN (g kg^{-1}ΣN)	132.6	137.6	138.7	139.2
Lactic acid (g kg^{-1} DM)	28.5	26.9	25.0	25.1
Acetic acid (g kg^{-1} DM)	13.7	13.2	13.3	13.7
Butyric acid (g kg^{-1} DM)	0.19	0.26	0.14	0.15
pH	4.8	4.8	4.8	4.8
Total fungi count (log10 CFU g^{-1})	2.11	2.71	2.73	3.4
ZEA (mg kg^{-1})	0.204	0.058	0.083	0.075
FB1 (mg kg^{-1})	3.425	3.615	5.140	6.600
DON (mg kg^{-1})	0.115	0.070	0.070	0.090

Application of N fertilizer has proven to be a negative agro-technical measure in the cultivation of grass-legume mixtures, with respect to the preparation of silage. Applied N reduces the ensiling ability of plants and silage quality. For these reasons, if during the cultivation of perennial crops, N fertilizer is used, it should be applied quantities that will have positive effect on the production characteristics of crops, but not greatly reduce the quality of the silage.

The influence of crop maturity in the stage of exploitation on the quality of grass-legume silage

Plants at each stage of growth and development are characterized by specific chemical characteristics. During ensiling, the plants should be exploited in the best phase of fermentable properties. Only in this way it is possible to get high quality silage.

Cutting in the later developmental stages significantly increases dry matter concentration, but reduces buffer capacity, concentration of crude protein, soluble carbohydrates and ash. However, in the silage, delayed cutting significantly reduces pH, buffer capacity, digestibility of dry matter, crude protein concentration, ammonia nitrogen, acetic and propionic acid and ethanol, and significantly increases the concentration of dry matter and fermentable sugars *(Keady and Kiely, 1998)*.

Also, plant growth stage at the time of exploitation affects the degradation of proteins as the prolonging the time of utilization and preparation of silage reduces protein degradability *(Kohn and Allen, 1995)*.

By preparing silage from grass biomass, which consisted of 80.6% cocksfoot, 13.7% legumes and 2.3% of other plants, in the late vegetative stage and

the start of flowering, *Vranić et al. (2008)* concluded that the DM content was lower in the vegetative stage, unlike the crude protein content that declined from the vegetative to the generative phase. Silage from the vegetative phase has fermented well with ammonia nitrogen content below 100 g kg^{-1} ΣN (78), while the silage from the beginning of flowering stage had ammonia nitrogen content higher than 100 g kg^{-1} ΣN (128.6). The pH was significantly different in the two stages of silage preparation. In the second phase, the pH value was higher (4.7) than the pH value of the first stage (4.4). Acetic acid was higher in the second (36.9), compared to the first stage of preparation of 1.2 g kg^{-1} DM, and there was no butyric acid.

In the study of the quality of silage grass-legume mixtures *Bijelić (2009a)* concluded that phenophase when the plants are utilized has highly significant effect on the dry matter, crude protein, ammonia and soluble nitrogen and lactic and acetic acids. The concentration of butyric acid and pH were not significantly changed depending on the phase of utilization of plants.

Table 5. Effect of crop maturity on the parameters of quality grass-legume silage *(source Bijelić, 2009a)*

Treatments	Beginning of flowering	50% of blooming
Dry matter (g kg^{-1})	332.6	353.5
Crude proteins (g kg^{-1} DM)	157.7	141.3
NH_3N in ΣN (g kg^{-1}ΣN)	141.6	132.4
Soluble N u ΣN (g kg^{-1}ΣN)	578.3	574.9
Lactic acid (g kg^{-1} DM)	26.9	27.1
Acetic acid (g kg^{-1} DM)	14.3	12.9
Butyric acid (g kg^{-1} DM)	0.09	0.28
pH	4.8	4.8
Total fungi count (log10 CFU g^{-1})	2.57	2.9
ZEA (mg kg^{-1})	0.1215	0.0895
FB1 (mg kg^{-1})	5.8300	3.5500
DON (mg kg^{-1})	0.0700	0.1050

The dry matter content in the later stages of exploitation increased as well as the content of lactic and butyric acid, and the contents of crude protein, ammonia and soluble nitrogen and acetic acid decreased. According to *Djordjevic (2000)* also the content of crude protein and ammonia nitrogen decreased, but the content of soluble nitrogen increased. Contrary to these studies, some authors state that the content of organic acids in silage decreases at later pheno-phases stages of growth and development of plants *(Kuoppala et al., 2007)* and that the content of lactic and acetic is reduced, and butyric acid increased *(Dawson et al., 2002)*. The results obtained by *Bijelić (2009a)* could be explained by the positive correlation between pheno-phase and content of fermentable sugars *(Henderson, 1973)*, as well as delayed clostridial fermentation.

Conclusion

Grass-legume mixtures are ideal for the preparation of silage. The fodder rich in nutrients necessary for successful livestock production is produced from grass-legume mixtures. Legume component increases protein and vitamin contents, while grass increases the content of easily soluble carbohydrates and minerals. For these reasons it is important to determine the structure of the compounds and determine the appropriate proportion of individual species in it in order to get enough balanced quality fodder. In addition to belonging to the botanical species, there are several factors that affect the quality of the silage. N fertilization is agro-technical measure used to achieve increase in the yield of crops, however it has a negative effect on the fermentable ability of plants and silage quality because it increases the buffer capacity, total nitrogen and nitrate contents, and reduces the content of fermentable sugars, favours the development of harmful microorganisms and the production of mycotoxins. Ensiling of crops should be done in conditions/phase of the best of fermentable ability. Cutting and ensiling in the later pheno-phases, stages of growth and development, significantly reduce pH, buffer capacity, digestibility of dry matter, concentrations of crude protein, ammonia nitrogen, acetic and propionic acid and ethanol, and significantly increase the concentration of dry matter and fermentable sugars.

Based on the fore mentioned, it can be concluded that the preparation of silage from grass-legume mixture is very important in modern livestock production. This method provides a highly valuable quality fodder, rich in protein, carbohydrates, minerals and vitamins, whose use is especially important in the winter months. In order to provide animals with a complete and quality diet, great attention should be paid to the proper selection of species for the formation of mixtures, proper fertilization, with appropriate quantities and in a timely manner, and optimum time of utilization.

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Travno-leguminozne silaže

Z. Bijelić, Z. Tomić, D.Ružić-Muslić, V. Mandić, A. Simić G. Marinkov, Lj. Stojanović

Rezime

Cilj rada je da se prikaže mogućnosti pripreme silaže od travno-leguminoznih smeša shodno njihovim fermentabilnim karakteristikama, faktorima koji utiču na kvalitet silaže direktno ili indirektno sa posebnim osvrtom na uticaj botaničke pripadnosti vrste i agrotehnike, đubrenja azotom i faze zrelosti useva u momentu iskorišćavanja. Biljne vrste koje se koriste za sastavljanje krmnih smeša odlikuju se različitim hemijskim i fermentabilnim karakteristikama. Stoga, da bi dobili silažu koja po svojim karateristikama zadovoljava zadate potrebe za hranljivim materijama, velika pažnja se mora pokloniti pravilnom odabiru vrsta za sastavljanje smeša. Pored strukture smeše, na kvalitet fermentacije i hemijske osobenosti silaže utiču i primenjene agrotehničke mere u toku vegetacije. Đubrenje azotom iako ima povoljnog uticaja na prinos biljaka dosta negativno utiče na fermentaciju i kvalitet silaže jer povećava puferni kapaciteta a smanjuje sadržaja fermentabilnih šećera. Biljke u različitim fenofazama, odlikuju se različitim hemijskim karakteristikama i fermentabilnim sposobnostima. Iskorišćavanje useva u optimalnim fazama razvića može nam obezbediti proizvod visokog kvaliteta.

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EFFECT OF DROUGHT AND FOLIAR FERTILIZATION ON MAIZE PRODUCTION

V. Mandić[1], A. Simić[2], Z. Tomić[1], V. Krnjaja[1], Z. Bijelić[1], G. Marinkov[1], Lj. Stojanović[1]

[1]Institute for Animal Husbandry, Autoput 16, 11080 Belgrade, Republic of Serbia
[2]University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade, Republic of Serbia
Corresponding author: violeta_randjelovic@yahoo.com
Invated paper

Abstract: According to its economic importance maize is a major cereal crop plant in Republic of Serbia. The average maize is grown on an area of 1.2 million hectares with a total annual production of about 6 million tons and an average grain yield of 4.9 t ha^{-1}. Of the total production of maize, 80% is used as animal feed. However, grain yield varies considerably due to the occurrence of stress caused by drought and high temperatures at flowering and grain filling. Proper and timely implementation of agro-technical measures can mitigate the effects of drought. Similarly, the application of mineral fertilizer through the leaves (foliar), when the adoption of nutrients through the roots is restricted in drought conditions, it is possible to increase the production of maize. In this way it is possible to provide a higher and more stable production at the farm.

Key words: drought, foliar fertilization, flowering, grain filling, maize

Introduction

Maize (*Zea mays* L.) is the most important cereal crop in Republic Serbia. All parts of the crop can be used for food and non-food products. This is a multipurpose crop, provides food and fuel for humans and feed for animals. Maize is refined to generate a wide range of products including maize oil, maize starch, sweeteners, beverage and industrial alcohol, bioethanol as fuel, and new byproducts such as aminoacids, antibiotics and degradable plastics are increasingly being synthesized using maize as a raw material. However, in Serbia maize is largely used as animal fodder (80% of the total production). Maize is widely used in animal nutrition mainly as grain and silage. Grain is primarily used for pig, horses and poultry feeding, while silage is important for ruminant nutrition (cattle, goats and sheep). Maize grain contains about 80% carbohydrates, 9 - 10% protein, and 4 - 5% oil, and is therefore an important energy source in the diet of local people and animals, and important raw material for industrial use. On the other

hand, maize is the most important forage crop in our country due to the high production of green mass per unit area (12-25 t total dry matter per hectare), high energy content of dry matter, convenience and quality of biomass for silage.

World, USA and Serbia maize production statistics

In regard to total planted area maize is the third in the world after wheat and rice, in regard to the total production maize takes the second place, and in regard to grain yield per unit area the first place. Average harvested area, total production and grain yield of maize worldwide for the 2007-2011 periods was the 162.8 million hectares, 834.7 million tones and 5.1 t ha^{-1}, respectively *(FAOSTAT, 2011)*, Table 1 and Figure 1. Maize is grown mainly from 50° latitude N to 40°S, at elevations ranging from 0 to 4000 meters above sea level. At higher latitudes, up to 58°N, it can be grown for silage. The USA is the largest producer of maize in the world, yielding about 38.4% of the total world production. Average harvested area, total production and grain yield of maize in the USA for the 2007-2011 periods was the 33.2 million hectares, 320.2 million tones and 9.6 t ha^{-1}, respectively.

Table 1. Harvested area and production in maize in world, USA and Serbia, 2007-2011 ***(FAOSTAT, 2011,*** *Statistical Yearbook of the Republic of Serbia, 2010, 2012)*

Year	Harvested area, ha			Production, t		
	World	USA	Serbia	World	USA	Serbia
2007	158.310.323	35.013.800	1.201.832	789.927.060	331.175.000	3.904.825
2008	162.387.781	31.796.500	1.273.908	829.104.646	307.142.000	6.158.122
2009	158.874.730	32.168.800	1.208.640	820.539.197	332.549.000	6.396.262
2010	164.069.689	32.960.400	1.229.573	850.445.143	316.165.000	7.207.191
2011	170.398.070	33.986.300	1.258.437	883.460.240	313.918.000	6.479.564
M	162.808.119	33.185.160	1.234.478	834.695.257	320.189.800	6.029.193
Index, %	100.0	20.4	0.8	100.0	38.4	0.7

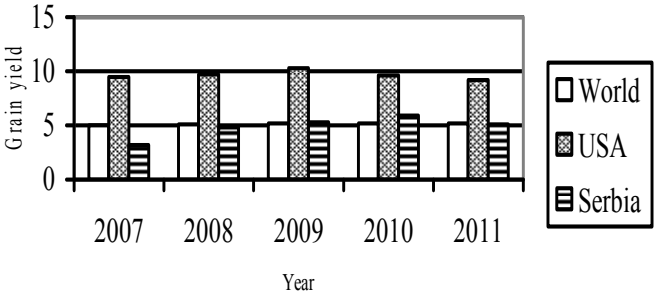

Figure 1. Grain yields in maize in world, USA and Serbia, 2007-2011 (t ha-1) ***(FAOSTAT, 2011,*** *Statistical Yearbook of the Republic of Serbia, 2010, 2012)*

Maize harvested area in Serbia for the 2007-2011 period was in mean 1.234.478 ha with variation among years in range from 1.201.832 ha (2007) to 1.273.908 ha (2008) *(Statistical Yearbook of the Republic of Serbia, 2010, 2012)*. The maize production for the 2007-2011 period were 6.029.193 t, range of variation from 3.904.825 t (2007) to 7.207.191 t (2010). The average grain yields for the same period were 4.9 t ha^{-1}, range of variation among the years from 3.2 t ha^{-1} (2007) to 5.9 t ha^{-1} (2010). Average grain yield of maize in Serbia is lower for 4.7 t (92.1%) than average grain yield of maize in USA. Rainfall and temperature regimes are most responsible factors for these yield differences. High variability of the rainfal regime is typical in Serbia during summer seasons. The year 2007 was the most unfavourable year for maize production *(Mandić, 2011)*.

Effects of drought on maize production

Effects of drought during flowering and grain filling. In climatic conditions for Serbia, 490 mm of rainfall (April 50mm, May 75 mm, June 90 mm, Jul 100 mm, August 95 mm, and September 80 mm) is necessary to meet the needs of evapotranspiration during the growing period *(Glamočlija, 2012)*. *Radičević et al. (2011)* reported that drought in Serbia in the period 1990-2010 occurred in the period from tasseling to milky maturity of maize and the most endangered were medium-late and early hybrids. *Ranđelović (2009)* and *Jeličić et al. (2004)* reported that maize under dry land farming needs at least 200 mm of water in June, July and August, and most of it in July. *Kovačević et al. (2009)* and *Maklenović et al. (2009)* pointed that the higher and good distributed rainfalls as well as upper air temperatures during June, July and August are mainly associated with high yields of maize. In the climatic conditions of Vojvodina, maize yield decreases linearly with the lack of water in July and August when it is in the stages of flowering and grain filling *(Bošnjak and Pejić, 1997)*. *Marković and Jovanović (2011)* concluded that in some municipalities of Vojvodina for the period 1975-2005, the highest correlation between the average maize yield and rainfall was in July. The correlation was much weaker before June and from September. *Pejić et al. (2009)* indicated that in Rimski Šančevi there was a high significant correlation between maize yield and amount of rainfall in August. *Ranđelović et al. (2011)* reported that weather events in 2006 and 2007, have a significant effect on the dry matter yield, grain yield, grain starch content, grain protein content, starch and protein yield in maize hybrids ZP 434, NS 444, ZP 684, NS 6010, ZP 735 and Dunav. Two years, 2006 and 2007, differed significantly in distribution of precipitation. In 2006, values for all studied traits were significantly higher then in 2007 because of higher amount and distribution of precipitation, especially during of June to August (Table 2). Maize is particularly sensitive to water stress from 1 week before to 2 weeks

after flowering *(Campos et al., 2004)*, 5 days before and 5 days after silking *(Shaw, 1974)*, 2 weeks before silking and 2-3 weeks after silking *(Frey, 1982)*. *Zinselmeier et al. (1999)* stated that the maize is particularly sensitive to water deficit 5 days before flowering for drought adversely affects fertility, and thus reduces the number of formed grains on the ear. *Monneveux et al. (2008)* concluded that the maize is sensitive to water deficits and high temperatures at flowering because anthers and silks are separated by about 1 m, and pollen and stigmas are exposed to the environment. Differences in grain yield were related primarily to differences in amounts and temporal distribution of rainfall during vegetation season, especially during Anthesis – Silk Interval - ASI *(Ranđelović et al., 2010a)*. The authors note that in our weather climate condition, ASI period in maize is mainly during the month of July, when usually there is not enough rainfall. The results showed that 1 mm of rainfall in the ASI would increase the grain yield of 61.8 kg ha^{-1}.

Table 2. Monthly air temperature (^{o}C) and total precipitation (mm) in 2006 and 2007 with long-term precipitation means (1961-1990) *(Ranđelović et al., 2011)*

Months	Average temperatures			Total of precipitation		
	1961-1990	2006	2007	1961-1990	2006	2007
October - March	-	-	-	258.1	212	256
April	11.5	12.6	13.0	51.1	63	0
May	16.5	16.4	18.5	58.2	32	79
June	19.3	19.6	22.1	84.3	92	86
July	20.7	22.7	22.6	64.6	39	39
August	20.2	19.2	22.2	54.2	157	63
September	16.5	17.5	14.3	44.1	16	94
Mean	17.4	18.0	18.9	-	-	-
Growing season	-	-	-	356.5	399	361
Total	-	-	-	614.6	611	617

Marković et al. (2008) found that drought conditions in maize lead to increase in the length of ASI period and a decrease in grain yield. Also, *Bolaños and Edmeades (1993)* reported that in drought conditions silk growth is delayed, leading to an increase the ASI. This leads to the delay of silk emergence, the reduction of pollen viability, the number of grain fertilized per ear is reduced. *Calvino et al. (2003)* stated that the grain yield of maize in Argentina ranges from 4.2 to 10 t ha^{-1} and predominantly (> 84%) depends on the amount of rainfall in the ASI period. If drought occurs after tasseling stage, ASI interval is extended *(Herrero and Johnson, 1981)* and thus influences the reduction of the grain filling period *(Westgate, 1994)*. *Grbeša et al. (2012)* concluded that drought reduces silage yield by 50-90%, especially if it occurs two days before and after silking and pollination leading to an undeveloped ear, occurrence of barren plants or plants with ear with few grain. In favorable years for growing maize, at the time of

harvest, ratio between maize grain and with leaves is 52% : 48%. In drought conditions, the share of stems increases. In drought conditions 1 t of silage contains 120 kg of grain, and in good years 182 kg. *Mandić (2011)* found that under drought conditions, a significant decrease occurs in plant height, stem diameter, ear length, number of grains per ear, grain weight per ear, 1000 grain weight and grain yield which reduces yield of silage.

Generally, it can be concluded that the reduction of the formed maize grain on the ear is the result of water deficit in the silking period - the beginning of grain filling. Water deficit in 2-3 weeks after flowering significantly reduces 1000-grain weight. All this contributes to a reduction in grain yield per unit area. In Serbia, a critical period for water is in July, but the negative effect shows also drought in August (the period of grain filling). Under drought - stress condition, the plant height, ear weight and the share grain in ear was reduced which leads to a reduction in the yield of silage and quality.

Effect of drought prior to tassel initiation on maize. On the other hand, *Abrecht and Carberry (1993)* have concluded that extreme drought in the beginning of the growing period does not affect grain yield and number of grain per plant. *Randjelović (2011)* established that extreme drought in the beginning of the growing period does not affect the germination of maize when agro-technical measures rolling of planting area is applied. By rolling the land is flattened, compacted, and physical contact between seed and soil established, providing the way of ascending movement of capillary water from deeper layers, breaking up lumps and crusts, provoking the weeds to sprout. Also, *Doorenbos and Kassam (1979)* have found that maize is more resistant to water deficit at the beginning and end of the growing season than in the flowering period. *Pandey et al. (2000)* have stated that maize plant reimburse the water shortage in the vegetative stage through the root system, which adopts water from deeper soil layers.

Technology of maize growing to mitigate the effects of drought. Safe way to obtain high and stable yields of maize in Serbia in the changing climatic conditions is the introduction of irrigation as well as agro-technical measures. However, the irrigation system in Serbia is installed on about 180.000 ha, of which 30.000-40.000 ha are irrigated, representing less than 1% of arable land. In order to achieve higher yields of maize it is necessary to apply the entire agricultural practices and production technologies in optimal time *(Maksimović et al., 2004)*. Results *Starčević et al. (1995)* showed that the proper and timely implementation of agro-technical measures can reduce up to 30% of the adverse effects of drought. Alternative agro-technical measures to mitigate the effects of drought, according to *Molnar et al. (2001)* are crop rotation, preceding crop, sowing structure, soil tillage, fertilization, mulching, the choice of genotype, time of sowing, plant density, weed control and windbreaks.

The role of foliar fertilization on maize production

In all systems of maize production, plant mineral nutrition is one of the main agro-technical measures, which to a large extent affects yield. Highly concentrated mineral nutrients NPK applied through soil and/or leaf and high-yielding genotypes provide great opportunities for more intensive and more cost-efficient production of field crops. Adoption of nutrients through the leaf surface is provided by the cuticle, stomata (essentially through the cuticle stomata) and ectodesms *(Witwer, 1963)*. Foliar absorption is most easily done through the epidermal cells of the leaf venation, leaf hairs, anticlinal walls of epidermal cells and stomatal guard cells, or places that have a large number of ectodesms *(Franke, 1961)*. Foliar fertilization is a supplement to the existing mineral nutrition, which has a direct impact on traffic of matters in the cell *(Kovačević, 2003)*. The fertilizer application on the soil is very dependent on rainfall, with rainfall that becomes the most important factor influencing crop production. Foliar fertilization is way for plants to provide the necessary macro-and micronutrients in the moment when it comes to disruptions in the rhythm of their adoption from the soil solution, caused by the lack of water *(Ranđelović, 2009)* or increased salt content *(Römheld and El-Fouly, 1999)*. For foliar fertilization to be effective it is necessary to have sufficiently large leaf area *(Ling and Silberbush, 2002)*. The authors have found that foliar fertilization of maize increases leaf area, dry matter content, chlorophyll, N, P and K in the leaves. *Nešić et al. (1973)* have recommended that foliar fertilization of maize using Wuxal should be conducted at the tasseling stage when the leaf area of plants coveres active area of growing over 30%. *Krzysch (1958)* pointed out that passing the nitrogen through the leaf is better and faster than taking the other elements. He concluded that in fruits, vegetables and wide row crops max. 5 - 30 kg ha^{-1} of urea can be administrated through leaves (2.5 to 15 kg of pure nitrogen per hectare). *Drezgić et al. (1979)* have concluded that foliar nitrogen application significantly increases grain yield and percentage of total protein in the grain. *Pavlov and Kolesnik (1965)* have concluded that foliar fertilization using carbamide (10-15 kg N ha^{-1}) increases protein content in maize leaves by 16-30%, in the stem by 15-41% and by 8-12% in grain. *Jelenić et al. (1972)* have concluded that foliar nutrition with nitrogen at the stage of tasseling of maize increased total protein content of 55% in grain of opaque-2 form, and 17% in the normal grain form. *Harder et al. (1982)* in the first year of their studies have concluded that foliar fertilization in drought conditions applied four weeks after silking had no significant effect on grain yield and yield components. In the second year of study, a foliar fertilization was applied two weeks after silking and a significant reduction in grain yield, 1000 grain weight and grain weight per ear was observed. However, decrease of values of these traits is the result of severe droughts during silking.

Foliar fertilization increased the nitrogen content of the grain by 10% and phosphorus by 4.7%. *Giskin and Efron (1986)* have treated foliarly maize plant in the 4-5 leaf stage with a nutrient containing N, P, K and S. In the first year they got higher yield of grain by 16.6% compared to the control, while the yield of silage did not differ. In the second year, grain and silage yields were higher in the treatment with foliar nutrition than in the control. The authors suggested that the maize for silage is treated in two foliar applications, and single treatment for maize grain. *Yuncai et al. (1991; 2008)* have concluded that foliar fertilization of maize reduces transpiration coefficient in terms of short-term drought and increased salt content in the soil, but does not significantly increase the growth of plants. *Mallarino et al. (2001)* have stated that foliar fertilization of plants at the beginning of the growing season, when the root has not been developed, increases supplies of phosphorus and potassium. *Tejada and Gonzalez (2006),* in the foliar treatment of maize plant with byproducts of beet vinasse (containing organic matter, N and K) obtained higher protein content by 30%, the number of grains per ear by 20% and grain yield by 13% compared to control. *Ranđelović (2009)* and *Ranđelović et al. (2010b)* have concluded that grain yield of *stay green* maize hybrids (suitable for the production of grain and silage) greatly depends on the type of foliar nutrients. Thus, foliar nutrition with Ferticare had a greater effect on yield than Humikal universal, although both increased significantly compared to the control (Table 3). The authors have made a recommendation that supplemental foliar fertilization of maize should be carried out at phase of 7-10 leaves in two applications Ferticare I. The same results were also obtained by *Prijić et al. (2003)* and *Cvijanović et al. (2004)*. The reason for this is that Ferticare I has a higher content of macronutrients N, P and K (14:11:25) than Humikal universal (4:4:4).

Table 3. Grain yield of maize - hybrid ZP 735, t ha^{-1} *(Ranđelović, 2009)*

Year	Variety of mineral nutrition (B)	Row (A)				M	Index (%)
		Border	Indeks (%)	Inner	Indeks (%)		
2003.	1. K	10.41	100.00	9.02	100.00	9.72	100.00
	2. P 5	10.67	102.50	9.32	103.32	10.00	102.88
	3. P 5 + H	10.78	103.55	9.42	104.43	10.10	103.91
	4. P 5 + F I	11.43	109.80	10.47	116.08	10.95	112.65
	M	10.82	-	9.56	-	10.19	-
	Index (%)	100.00	-	88.35	-	100.00	-
2004.	1. K	10.63	100.00	10.10	100.00	10.37	100.00
	2. P 5	11.22	105.55	10.97	108.61	11.10	107.04
	3. P 5 + H	11.59	109.03	11.06	109.50	11.32	109.16
	4. P 5 + F I	12.14	114.20	12.01	118.91	12.08	116.49
	M	11.40	-	11.03	-	11.22	-
	Index (%)	100.00	-	96.75	-	110.11	-
M	1. K	10.52	100.00	9.56	100.00	10.04	100.00
	2. P 5	10.94	103.99	10.14	106.07	10.54	104.98
	3. P 5 + H	11.19	106.37	10.24	107.11	10.72	106.77
	4. P 5 + F I	11.79	112.07	11.24	117.57	11.52	114.74
	M	11.11	-	10.30	-	10.70	-
	Index (%)	100.00	-	92.71	-	-	-

Legend: K - control; P 5 - Power 5; P 5 + H - Power 5 + Humikal univerzal; P 5 + F I - Power 5 + Ferticare I

LSD	2003 year			2004 year		
	A	B	A*B	A	B	A*B
$LSD_{0.05}$	0.56	0.79	1.10	0.048	0.068	0.095
$LSD_{0.01}$	0.76	1.07	1.49	0.065	0.092	0.129

The importance of foliar application of Ferticare I on grain yield has been indicated in the research of *Ranđelović et al. (2006; 2009b)*. The authors recommended that foliar fertilization should be introduced in the production of soybean because soy is grown mainly in the dry farming system and during the growing season crops are exhibited to a number of stress factors. *Radulov et al. (2010)* have concluded that foliar fertilization of maize with fertilizers containing different amounts of N, P and K, increases protein content and changes the ratio of amino acids in crude protein. *Mandić (2011)* found that foliar fertilization of *stay green* hybrids (NS 6010, ZP 684, and Dijamant-6) with Slavol significantly reduces the number of lodged plants and number of plants without ear and significantly increases plant height, ear height, stem diameter, number of leaves per plant, ear length, number of grain per row, number of grains per ear, grain weight per ear, 1000-grain weight, ear diameter, cob diameter, rachis diameter and grain yield. Due to the increase in property values that affect the yield of silage, the

author concluded that foliar feeding has a positive impact on increasing the yield of biomass for silage.

Generally, foliar fertilization can eliminate lack of macro-and micronutrients, especially slow moving or nearly stationary elements (Ca, Fe, B). They should be applied in circumstances where the adoption of nutrients from the soil solution is limited due to long-term drought, high or low temperatures of soil, unfavorable pH (antagonism or inactivation of ions at high (Fe, Mg, Mn, B, C) or low pH values (P, Mo , Mg)), nutrient leaching from arable layer, etc.. Foliar treatment of plants can be expected to increase the grain yield due to a reduction in the percentage of barren plants, increased ear length, number of grain per row, number of grain per ear, grain weight per ear and 1000 grain weight. The increase of the yield of silage can be expected due to the increased plant height, stem diameter, number of leaves per plant, leaf area, ear length, number of grain per ear, grain weight per ear and 1000 grain weight. Since there is a decrease in the share of ear rachis portion in ear mass, one can expect improved quality of silage. Similarly, foliar treatment plants can be expected to improve the quality of grain and silage because it leads to an increase in total protein content and amino acid changes in the proportions of grain and increase the protein content in the leaf and stem. Due to the reduction of production costs foliar fertilizers should be used together with plant protection products.

Growing of maize in the system of foliar nutrients application, and for the production of animal feed is justified because it gets higher yields of grain and silage. On small rural farms this method of maize growing can be applied in the production practice with the application of existing machinery with the minimum investment. In this way it would meet their needs and the need for greater amounts of feed and round up the entire production on the farm, or links between farming and animal husbandry.

Conclusion

Stable agricultural production of maize in Serbia is limited by drought. Drought usually occurs in the summer months (June, July, August) as a result of the high air temperature, low relative humidity, high evapotranspiration and insufficient rainfall. The negative effects of drought can be mitigated by applying foliar fertilizers because adoption/intake of nutritious substances is not restricted by drought land as opposed to the root. System of foliar fertilization of maize should be extensively studied and linked to the system of production, soil properties and climate, economic and other factors important for a high, stable and economical production.

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Efekat suše i folijarne ishrane na produkciju kukuruza

Rezime

V. Mandić, A. Simić, Z. Tomić, V. Krnjaja, Z. Bijelić, G. Marinkov, Lj. Stojanović

Po svom privrednom značaju kukuruz je jedna od najvažnijih ratarskih biljaka u Republici Srbiji. U proseku kukuruz se gaji na površini od oko 1.2 miliona hektara sa ukupnom godišnjom proizvodnjom od oko 6 miliona tona i prosečnim prinosom od 4.9 t ha^{-1}. Od ukupne prozvodnje kukuruza 80% se koristi u ishrani domaćih životinja. Međutim, prinos zrna kukuruza značajno varira usled pojave stresa izazvanog sušom i visokim temperaturama u fazi cvetanja i nalivanja zrna. Pravilnom i blagovremenom primenom svih agrotehničkih mera moguće je ublažiti efekat suše. Isto tako, primenom mineralnih hraniva preko lista (folijarno), kada je usvajanje hranljivih materija preko korena ograničeno u uslovima suše, moguće je povećati produkciju kukuruza. Na ovaj način moguće je osigurati njegovu veću i stabilnu proizvodnju na gazdinstvu.

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ORALLY PRESENTED PAPERS

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COMPARISON OF AD LIBITUM AND RESTRICTIVE FEEDING OF FATTENING PIGS

K. Fischer, U. Wuestemann, H. Schlegel, M. Waehner

Anhalt University of Applied Sciences, Strenzfelder Allee 28, 06406 Bernburg, Germany
Corresponding author: k.fischer@loel.hs-anhalt.de
Original scientific paper

Abstract: The optimal feeding of fattening pigs is essential for a successful meat production. In an experiment we compared ad libitum and restrictive feeding of female and castrated pigs. The pigs were kept in a commercial farm with liquid feeding. For investigation in total 20 pigs of BHZP genetic have been used, 10 in each feeding method and 5 castrated males and 5 females for each feeding. All pigs have been weighed once a week. In result castrated males achieve with 893g per day best results for daily weight gain in fattening period with ad libitum feeding compared to 717g per day with restricted feeding. Lowest results were achieved by female pigs with 705g per day with ad libitum feeding in comparison to 766g per day with restricted feeding. Noticeable are the high variations between the pigs in each group. In the groups of ad libitum feeding there are differences in daily weight gain of 200g per day. Comprehensible there are lower differences in the group of restrictive feeding. One reason for low results of the pigs in the restrictive feeding group is the feeding curve of the farm. The estimated bodyweight to calculate the requirements of energy and protein have been too low in the first 6 weeks. The real weight was higher. In conclusion it is necessary to measure the weight of the pigs to adapt the feeding curve in restrictive feeding systems and it is possible to manage fattening pigs with ad libitum feeding until the end.

Key words: ad libitum feeding, restricted feeding, fattening pigs

Introduction

The way of feeding is an essential decision to produce finishers in an effective way. In experiments of BRANDE et al. (2009) comparisons of restrictive and ad libitum feeding have been done. It is possible to work with ad libitum feeding also in the third and last period for castrated and female pigs. Daily weight gain was 947g for castrated and 884g for female pigs was achieved only with ad libitum feeding.

Material and Methods

The experiment was conducted in a practical farm. They have several barns with ad libitum and restrictive feeding, so that it was possible to compare both systems in one farm at the same time. All pigs are of BHZP genetic. We selected 10 pigs for each feeding system, 5 castrated and 5 female ones. All selected animals got a special ear tag and have been weight once a week during the finishing period. All pigs are vaccinated against mykoplasmen and circovirus. There is no special vaccination at the beginning of the finishing period. For slaughtering the pigs got marketed at two different slaughter houses. In general the weight at the beginning is in average 27.6kg. The finishing period is divided into 3 parts. The first one lasts until an average weight of the pigs of 65kg, the second period lasts until a weight of 95kg and after that the third period begins. The adaptation of the feeding curve is done every Thursday. In figure 1 you can see the calculated weight of the pigs and the amount of consumed energy in MJ ME.

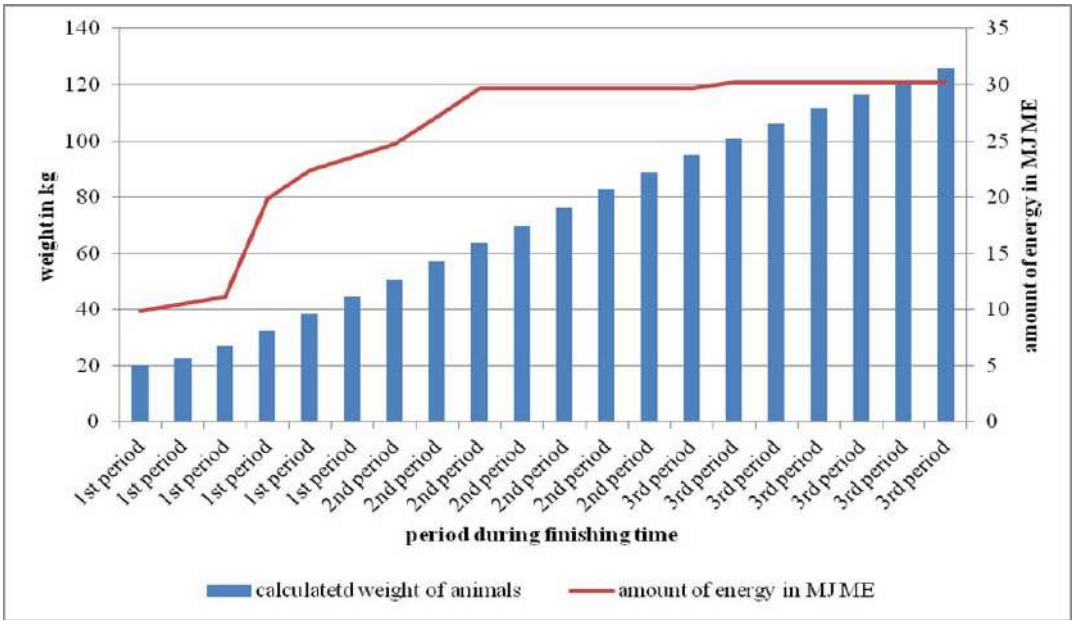

Figure 1: weight of animals and amount of energy in MJ ME according to the period during finishing

The following feeds are used wheat, barley, soybean meal and minerals. The mixture with water is 1 part of water and 3 parts of feedstuff. The exact mixtures in the 3 periods of finishing are shown in table 1.

Table 1. Rations of feeding in different periods of finishing pigs

amount	barley in %	wheat in %	soybean meal in %	mineral in %	energy in MJ ME	Crude protein in %
1st period	18.0	60.0	18.0	4.0	13.34	17.80
2nd period	24.5	55.0	17.5	3.0	13.36	17.29
3rd period	50.0	32.5	15.0	2.5	13.09	15.99

Depending on the number of pigs in one barn there are one or two drinking troughs. The experiment was conducted from 18th of June until the 20th of October 2012.

Results and Discussion

The average weight at the beginning of the finishing period was 27.6kg. Independent from the kind of feeding the female pigs have been lighter by 4 kg than the castrated ones. At the end of the finishing the average weight of the 20 fattening pigs was 109.2kg. The highest variation was detected within the group of castrated pigs with ad libitum feeding. The exact data are shown in table 2.

Table 2. Means and standard deviation of weight of castrated and female pigs according to the kind of feeding

	castrated pigs (n= 5)		female pigs (n= 5)	
	initial weight	slaughter weight	initial weight	slaughter weight
ad-libitum feeding	29.4 ± 3.5	118.0 ± 7.7	24.8 ± 1.6	102.8 ± 9.3
restrictive feeding	30.2 ± 3.7	106.4 ± 5.7	26.0 ± 2.9	109.6 ± 4.5

The average slaughter weight differed very much between feeding groups and sex of the pigs. The highest difference is detected in the group of ad libitum feeding between castrated and female pigs with 15.2 kg. Castrated pigs achieve highest results with 118kg and female ones the lowest results with 102.8kg.

The duration of fattening period was 120 days in maximum. Some animals got marketed earlier, because they reached the final weight. In average the pigs were kept for 113 days. Castrated males fed ad libitum have grown fastest. That's why they were kept only for 106 days. Those castrated males achieved a daily

weight gain of 893g per day. On second place there are female pigs in the group of restricted feeding with a daily weight gain of 766g per day. The exact data are shown in table 3.

Table 3. Daily weight gain in g per day according to sex and feeding regimen

	castrated males (n=5)	females (n=5)
ad libitum feeding	893 ± 80	705 ± 84
restrictive feeding	717 ± 55	766 ± 37

In general one can say that the performance level to low of castrated males in the group of restrictive feeding and of the females in the group of ad libitum feeding especially if you compare it to data from BRANDE et al. (2009). In that trial pigs fed ad libitum reached a daily weight gain of 947g for castrated and 884g for female pigs. Results from MEYER et al. (2011) are higher with 963g daily weight gain.

The variation within the parameter of daily weight gain is very high and so the time varies very much until the pigs reach their slaughter weight. As expected the differences are the highest in and between the groups of ad libitum feeding. The difference can reach up to 200g weight gain per day for female as also for castrated males. For restrictive feeding the results are more equal. The differences are only 96g weight gain per day for females and 129g weight gain per day for castrated males. In figure 2 those results and differences between the five animals per group of feeding and sex are demonstrated.

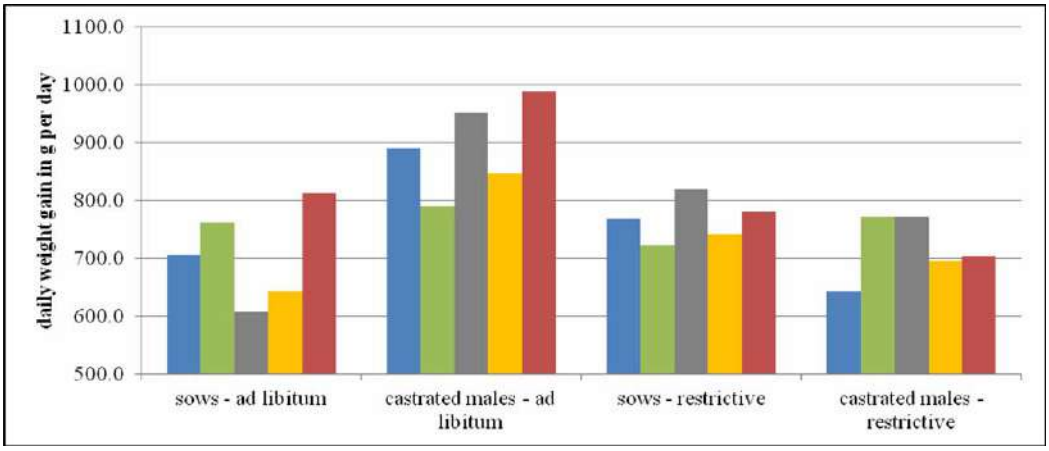

Figure 2. Daily weight gain in g per day according to sex and feeding regimen

The performance level of pigs in the group fed restrictive is to low especially for castrated males. One possible reason is the difference between the real weight of the pigs and the assumed one which they use to create the feeding curve. Picture 3 shows the big differences between those two parameters. At the beginning of the fattening period those animal do not get enough food. The assumed weights are too low. Even at the start of the fattening period the pigs already weigh about 27kg, but the feeding curve starts with 20kg of bodyweight. In consequence the pigs get less food and energy than they would need for growing. After 6 weeks the situation changes. From that moment on the assumed weights are higher than the real ones.

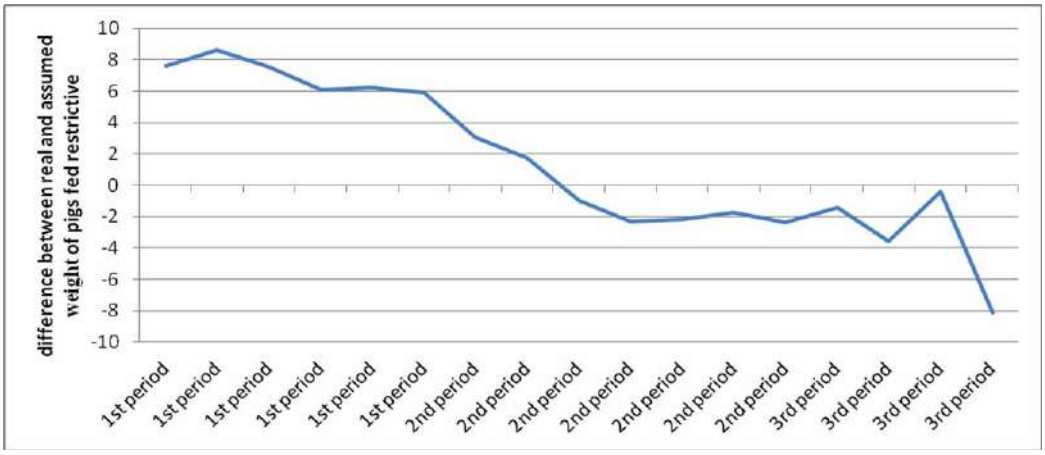

Figure 3. Difference between assumed and real weights of pigs fed restrictive

In addition two facts need be pointed out. First, the change to the third foodstuff is too late. The pigs already have a weight of 95kg. Second the amount of energy is limited to 30 MJ ME per animal and day. This should be increased up to 35 -36 MJ ME per pig and day (BRANDE et al., 2009; KLEINE-KLAUSING and RIEWENHERM, 2012).

In a subsequently experiment data of 224 slaughter pigs of this farm have been analyzed according to the regimen of feeding. The information on sex was not available. The following data have been documented slaughter weight, EUROP-classification, lean meat percentage, back fat thickness, meat thickness and reflectance value (table 4).

Table 4. Data of slaughter performance according to feeding regimen

	ad libitum feeding (n=169)	restrictive feeding (n=55)
slaughter weight (kg)	90.4 ± 6.5	90.0 ± 5.8
lean meat percentage (%)	56.7^{a} ± 3.3	57.8^{b} ± 2.6
back fat thickness (mm)	16.3 ± 3.4	15.4 ± 2.6
meat thickness (cm²)	59.3^{a} ± 7.7	61.7^{b} ± 6.6
reflectance value	34.3^{a} ± 4.0	35.8^{b} ± 3.2
S and E (%)	69	87
U (%)	30	11
R (%)	1	2

$^{a:b}$ $p<0.05$

The results on slaughter weight and back fat thickness do not differ between the two groups of feeding. According on the parameter of lean meat percentage, meat thickness and reflectance value pigs fed restrictive achieve significant better results. Because of those better results more pigs from the restrictive fed group are classified into group S or E (87%) at the slaughterhouse. From the slaughter pigs fed ad libitum the amount is less. Only 69% of those pigs have been classified into group S or E. The differences are not significant.

Conclusion

Two feeding system have been compared in a practical farm for castrated pigs and sows. In a first experiment 20 pigs have been weighed once a week during the whole fattening period. Data from the slaughterhouse have been analyzed in a second trial. In the fattening period the best results are achieved by castrated males which are fed ad libitum. For sows it is different. They achieve best result when they get fed restrictive. In this case the feeding curve needs to be optimized and adopted to the real weight of the pigs. Then it will be possible to achieve better results for castrated males also. Ad libitum feeding is possible but leads to more efforts in sorting the pigs for slaughterhouse, because they grow up very unequally. The results from slaughterhouse show advantages for pigs fed restrictive.

Poređenje *ad libitum* i restriktivne ishrane tovljenika

K. Fischer, U. Wuestemann, H. Schlegel, M. Waehner

Rezime

Optimalna ishrana tovljenika je od suštinske važnosti za uspešnu proizvodnju mesa. U eksperimentu smo poredili *ad libitum* i restriktivnu ishranu ženki i kastriranih svinja. Svinje su držane na komercijalnoj farmi sa tečnim sistemom hranjenja. Za ogled je korišćeno ukupno 20 svinja BHZP genetike, 10 u svakom metodu ishrane i 5 kastriranih mužjaka i 5 ženskih grla po svakom tretmanu ishrane. Sve svinje su merene jednom nedeljno. Kastrirani nerastovi postigli su sa 893g dnevno najbolje rezultate za dnevni prirast u periodu tova sa ishranom *ad libitum* u odnosu na 717g dnevno u sistemu sa restriktivnim hranjenjem. Najniži rezultati su evidentirani kod ženskih grla svinja sa 705g dnevno uz ishranu *ad libitum* u odnosu na 766g dnevno u sistemu sa restriktivnim hranjenjem. Primetno su visoke varijacije između svinja u svakoj grupi. U grupama sa ishranom *ad libitum*, postoje razlike u dnevnom prirastu od 200g dnevno. Očekivano postoje niže razlike u grupi restriktivne ishrane. Jedan od razloga za niske (slabije) rezultate svinja u grupi sa restriktivnom ishranom je kriva ishrane na farmi.Procenjene telesne težine za izračunavanje potreba u energiji i proteinima su bile isuviše niske u prvih 6 nedelja. Realna težina je veća. Može se zaključiti da je potrebno da se izmeri težina svinja kako bi se prilagodila krivoj ishrane u sistemu restriktivne ishrane i moguće je upravljati tovom svinja sa ishranom *ad libitum* do kraja.

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THE EFFECT OF FERMENTED MIXED COCONUT OIL AND TOFU SOLID WASTE SUPPLEMENT ON MEAT TENDERNESS AND CHOLESTEROL OF BROILER

H. A. W. Lengkey[1], F. R.wolayan[2], M. Najoan[2], P. Edianingsih[1], B. Bagau[2]

[1] Faculty of Animal Husbandry, Universitas Padjadjaran, Bandung, Indonesia
[2] Faculty of Animal Husbandry Sam Ratulangi University, Manado, Indonesia
Corresponding author: lengkeyhendronoto@gmail.com
Original scientific paper

Abstract: Study of the effect of Fermented Mixed Coconut Oil and Tofu Solid Waste Supplement on Meat Tenderness and Cholesterol of Broiler was aimed to determine the effect of the mixture of solid coconut oil and tofu waste that fermented with *Aspergillus niger* in ration on meat tenderness and meat cholesterol of broiler. Meat quality is one of final point of the consumer. There are some indicators such as meat tenderness and cholesterol contents. Solid coconut oil and solid tofu waste mixture and fermented by *Aspergillus niger* (CAKTAF) can be used as supplement in broiler feed. CAKTAF contained high protein and low fat; will improved the meat quality. 120 Cobb strain day-old chicks were used in this study for 5 weeks. The ration and drinking water was gave *ad-libitum*. This experiment used Completely Randomized Design (CRD) 6 x 4, consisted of six treatments (the usage rate of CAKTAF of 0% (R-0), 5% (R-1), 10% (R-2), 15% (R-3), 20% (R-4) and 25% (R-5) in the ration, and each treatment was repeated four times. Statistical tests performed by analysis of variance and differences between treatments effects were examined using Duncan's multiple range test. Results indicated that usage up to 25% CAKTAF in the ration will increase the meat tenderness and decrease the cholesterol contents in the meat.

Key words: *Aspergillus niger*, broiler, CAKTAF, fermentation, solid coconut oil waste, solid tofu waste.

Introduction

To avoid the competition between food and feed, we must seek the alternatives for feed ingredients because almost of the raw materials or components of feed are also used as human food. Agricultural processing wastes, such as solid

coconut oil dregs and waste tofu processing, can be utilized as raw material for animal feed rations constituent. The problem of using processing waste is the poor nutrition quality. The fermentation process can be applied to improve the quality of the waste; using microbes.

Meat tenderness may be influenced by changes occurring during muscle conversion to meat. These changes may be controlled, to improve meat quality. The influence of diet on meat properties is minor importance if there are no nutritional deficiencies. Any feeding practice which alters the quantity of glycogen stored in muscles can influence the ultimate meat properties. Some of the physical properties of fresh meat are difficult to measure objectively. Many factor within muscles, such as intra muscular fat content, can contribute to these physical properties. Tenderness is one of palatability factor that has received more research study (*Aberle, et al; 2001*). According to the laboratory and consumer studies, have shown that tenderness is the most important sensory attribute of meat. Tenderness and juiciness are closely related, the more tender the meat, the more quickly the juices are released by chewing it. The deposits of fat in muscle, add to the juiciness and flavor of meat, when it was cooked. Small amount of fat in feed have beneficial effects on the physical characteristics of the feed, and tends to influence the meat.

One aspect about feeding of fats to animals is tends to deposit the type of fat consumed in the ration. The saturated fat and cholesterol content of meat and meat products which are able to exert a definite increase in human serum lipid concentrations. Lipid content of meat is generally its most variable component. One of the lipid components of major concern from a nutritional standpoint is cholesterol. Attempts have been made to lower the cholesterol in eggs (*Elkin, 2007*) by feeding different grains to layers, which may reduce egg cholesterol by about 10 percent. Feeding copper at 125 or 250 parts per million (ppm) can reduce cholesterol in eggs by up to 31 percent. Feeding garlic as a paste at up to 8 percent of the diet may reduce egg cholesterol by as much as 24 percent, but there is wide variation. Other natural products have also shown significant but inconsistent responses. According to *Talat, et al (2000),* there are an average 62.20; 49.75; 58.44; and 41.80 mg/dl cholesterol contents in raw muscle of RIR, broiler, Fayoumi and cross birds, respectively.

One of the advantages in fermentations, the controlled action of selected microorganism is used to alter the texture, which increase the quality and value of raw materials. *Aspergillus niger* is a type of mold that is used commercially in improving the quality of agricultural processing wastes, because of the easy handling, its ability to grow quickly and it is not harmful because it does not produce mycotoxins. This fungus can produce enzymes such as a-amylase,

amylase, cellulase, gluco-amylase, catalase, pectinase, lipase, and galactosidase (*Ratledge, 1994*).

Material and Methods

Materials: Preparation of fermentation products:

- Coconut oil solid waste, from the coconut oil processing home industry (VCO) North Minahasa, North Sulawesi, Indonesia.
- Tofu solid waste, obtained from the Industri Pengolahan Tahu Cikuda, Sumedang; West Java, Indonesia
- Fungus *Aspergillus niger*, obtained from the Laboratory of Biological Sciences, Institut Teknologi Bandung; Indonesia
- Rice, fresh sprouts, gelatin, sugar, obtained from Pasar Suci, Bandung, Indonesia.

Experimental animal: 120 Cobb strain day-old chickens broiler from PT. Missouri Bandung, Indonesia.

Table 1. Composition of Rations Research (%)

Feed Contents	Treatments					
	R0	R1	R2	R3	R4	R5
Corn meal	56.44	52.44	48.44	44.44	40.44	36.44
Soybean meal	29	28	27	26	25	24
Fish meal	9	9	9	9	9	9
Coconut oil	2.5	2.5	2.5	2.5	2.5	2.5
Methionine	0.16	0.16	0.16	0.16	0.16	0.16
Top mix	0.5	0.5	0.5	0.5	0.5	0.5
CaCO3	0.9	0.9	0.9	0.9	0.9	0.9
Di-calcium phosphate	1.5	1.5	1.5	1.5	1.5	1.5
CAKTAF	0	5	10	15	20	25
Total	100	100	100	100	100	100

Note: Compiled by standard requirement by Lesson and Summer (2005)

From Table 1, the compositions are differs only in corn meal, soybean meal and CAKTAF.

Table 2. Standard ration and nutritional value of research diet

Feed Contents	Treatments					
	R0	R1	R2	R3	R4	R5
Protein (%)	22.56	22.49	22.43	22.37	22.31	22.25
Metabolizable Energy (Kcal/kg)	3023.97	3025.61	3027.25	3028.89	3030.53	3032.17
Fat (%)	6.45	6.41	6.36	6.31	6.28	6.22
Crude fiber (%)	3.40	4.44	4.32	4.40	4.41	4.41
Lysine (%)	1.33	1.33	1.33	1.33	1.32	1.32
Methionine (%)	0.60	0.59	0.59	0.59	0.59	0.59
Met + Cyst (%)	0.93	0.93	0.92	0.91	0.91	0.90
Ca (%)	1.08	1.09	1.09	1.09	1.09	1.09
P-non-phytate (%)	0.52	0.52	0.52	0.51	0.51	0.51

Rations: Rations used in the experiment consisted of yellow corn meal, fish meal, soybean meal, solid coconut oil dregs, DCP, Top Mix, CaCO3, and CAKTAF (the mixture of coconut pulp/coconut oil solid waste and tofu solid waste) fermented by *Aspergillus niger*. Feed materials were obtained from PT. Missouri Bandung.

Variables studied

Raw muscle samples were then analyzed for meat tenderness and meat cholesterol. The variables studied in the experiments are meat tenderness and meat cholesterol. The data thus collected was subjected to statistical analysis (Steel and Torrie, 1991) to test cholesterol contents in meat with Lieberman-Buchards method. The tenderness has been identified as the most important factor determining consumer eating satisfaction of meat with Universal penetrometer.

Research Design

This research used Completely Randomized Design (Steel and Torrie, 1991), with 6 treatments and each treatment was repeated 4 times, (there are 120 chickens for 24 experimental units, so each unit were 5 chickens).

Treatment rations were given as follows:
R0 = Ration containing 0% fermented products (CAKTAF) as control,
R1 = Ration containing 5% fermented products (CAKTAF),
R2 = Ration containing 10% fermented products (CAKTAF),
R3 = Ration containing 15% fermented products (CAKTAF),

R4 = Ration containing 20% fermented products (CAKTAF),
R5 = Ration containing 25% fermented products (CAKTAF).
Note: CAKTAF is fermented Coconut oil solid waste and Tofu solid waste.

Results and Discussion

The effect of treatment on meat tenderness

In Table 3, there are the results from using of CAKTAF (fermented solid waste of coconut oil and tofu) in ration, to the broiler carcass tenderness. The highest meat tenderness was get from the broiler that fed R-5 (diet + 25% CAKTAF (185.75 mm/g/10sec) and the lowest was get from the broiler that fed basal diet/0% CAKTAF, R-0 (132.75 mm/g/10sec).

From Table 3, the meat tenderness values were between 13.275 mm/g/10 sec to 18.575 mm/g/10 sec. Meat tenderness was significantly better in the groups which consumed CAKTAF. The tenderness will increase when the CAKCAF in the ration level percentage are higher. It means that the meat from broilers that given only ration without CAKTAF has the lowest tenderness. When given CAKTAF, the meat tenderness will increase. The more CAKTAF in the ration, will results the more tenderness of the meat. In R-0 (diet without CAKTAF-control), the tenderness are 13.275 mm/g/10sec, will increase when the level of CAKTAF more higher; in R-1(5% CAKTAF) 14.125 mm/g/10sec; and in R-2 (CAKTAF 10%) the tenderness is 14.925 mm/g/10sec; the R-3 (CAKTAF 15% in ration (15.325 mm/g/10sec); R-4 (20% CAKTAF) the tenderness 16.90 mm/g/sec; and in R-5 (CAKTAF 25%), has the highest tenderness (18.575 mm/g/sec). It means that the CAKTAF will influence the meat tenderness.

Table 3. The effect of treatment in ration on meat carcass tenderness (mm/g/10 sec)

Replication	R-0	R-1	R-2	R-3	R-4	R-5
I	12.60	12.10	12.80	13.20	15.90	19.50
II	12.40	14.50	14.20	13.40	16.80	17.10
III	13.60	15.20	15.50	15.40	16.00	16.60
IV	14.50	14.70	17.20	19.30	18.90	21.10
Average	13.275	14.125	14.925	15.325	16.90	18.575

According to *Aberle et al (2001)* the influence of diet on meat properties is minor importance if there are no nutritional deficiencies. The influence of diet on the physical properties of muscle, as long as no serious nutritional deficiencies, the feeding practice in ante mortem period which alters the quantity of glycogen stored in muscles can influence the ultimate physical properties of meat.

Table 4. Duncan's Multiple Range Test on meat tenderness during research

Treatments	Average Feed Consumption	Significancy (0.05)
R-0	13.275	a
R-1	14.125	b
R-2	14.925	b
R-3	15.325	b
R-4	16.900	bc
R-5	18.575	c

Note : The same letter in the significancy column showed no significancy

The CAKTAF influence the tenderness, because the fermented supplement (CAKTAF) that controlled the action of *Aspergillus niger* altered the texture which increase the quality and value of raw materials (solid oil and tofu waste). This material will alter the quantity of glycogen stored in muscles that will influence the ultimate meat properties (*Aberle et al, 2001*).

By Duncan test, results showed that the usage rates of CAKTAF (5%, 10%, 15%, 20% and 25%) in the rations raised the chicken meat tenderness; which were significantly different ($p < 0.05$), but the using of 25% CAKTAF has significant effect ($p < 0.05$), which were higher in comparison to other treatments (without CAKTAF- 0%; 5%, 10%, 15% and 20% CAKTAF). This facts illustrated that the addition of CAKTAF up to 25% level (R-1 till R-5) in ration, on meat tenderness were able to well respond to chewed the meat.

Meat tenderness of broilers was influenced by the supplement of feed ingredients that making up the ration. The 25% CAKTAF (R-5) tenderness was caused by the expandable nature of fermentation products used in the ration. The higher it expands, it will accelerate the tenderness of the meat.

The effect of treatment on meat cholesterol

The fat content of the rations are between 6.22 mg/100 g (R-5) to 6.45 mg/100 g (R-0). And the average of total cholesterol during 5 weeks of study, are between 39.99 mg/100 g to 38.74 mg/100 g. In Table 5, there are the results of total cholesterol content in broiler meat.

Table 5. Cholesterol content in Broiler meat (mg/100g)

Replication	Treatments					
	R-0	R-1	R-2	R-3	R-4	R-5
I	38.32	40.21	38.49	38.93	38.90	38.97
II	41.45	39.98	40.21	39.84	39.73	39.42
III	40.21	39.89	40.32	38.88	39.94	39.23
IV	39.98	38.98	39.90	39.44	38.32	37.32
Total	159.96	159.06	158.92	157.09	156.89	154.94
Average	39.99	39.77	39.73	39.27	39.22	38.74

The results of the average cholesterol content in broiler meat during the study (5 weeks) ranged from 39.99 mg/100g until 38.74 mg/100g, as seen in Table 5. The cholesterol content of raw and cooked meat and poultry products ranges from 40 to 90 mg/100 g (*Dinh, et. al., 2011*). So the cholesterol values in this study were lower than the normal cholesterol content. According to *Skrivan, et al (2002*), total lipid and cholesterol levels in breast muscle were suppressed by copper significantly in group 3 by 30% and 20%. Their results showed that dietary copper supplementation alters lipid metabolism and changes the fatty acid composition. The cholesterol content in this study will decrease in the meat that feeding CAKTAF in the ration. Then the differences of the treatment on cholesterol content in broiler meat were analyzed. Results show that the ration which contents of fermentation products (CAKTAF) showed significance ($p < 0.05$) on cholesterol content of broiler meat. And then, by Duncan multiple range test to find out the differences between treatments are shown in Table 6.

Table 6. Duncan's Multiple Range Test on meat cholesterol content

Treatments	Average Meat Cholesterol	Significancy (0.05)
R0	39.99	a
R1	39.77	a
R2	39.73	a
R3	39.27	a
R4	39.22	a
R5	38.74	b

Note: The same letter in the significancy column showed no significant difference

By Duncan tests, showed that the cholesterol content of broiler meat, feeding of R-1, R-2, R-3, R-4 and R-5 decreased compared to R-0, but R-1, R-2, R-3, and R-4 has significancy ($p < 0.05$) than R-5. It means that CAKTAF (5%, 10%, 15% and 20%) in the diet has no significancy on cholesterol content of broilers meat ($p < 0.05$) compared to R-0, but 25% CAKTAF in ration (R-5) showed significant difference ($p < 0.05$); that decreased the cholesterol content compared to the ration 0% CAKTAF (control) and rations contain CAKTAF of 5%, 10%, 15%, and 20%. This illustrated that the addition until 20% CAKTAF in the ration, still useful on cholesterol content of broiler meat.

These results are supported to the improvement on nutritional value, especially on coconut and tofu dregs fermented mixture. In accordance with the facts found in the first and second phase of the research; that the protein content increased, but decreased the crude fiber and crude fat, and the metabolizable energy value and protein digestibility increased compared with CAKTAF ration. The fermentation process by *Aspergillus niger* is able to break down the fats into

smaller compounds in the form of fatty acids, was easily absorbed by broiler chickens.

The low cholesterol contents of broiler meat that fed ration content 25% CAKTAF is caused by the reduced of fat in the ration, and influenced the cholesterol metabolism. Also, according to *Nousiainen and Setala (1998),* the intestinal flora contributes to lipid metabolism of the host in two different ways; first, bacteria can digest dietary and endogenous lipids by lipases and hydrogenate the free fatty acids, and second they can de-conjugate bile acids and modify cholesterol metabolism. It means that the *Aspergillus niger* has an effect to the cholesterol content in the meat that feed CAKTAF. These results are also lower than the results of *Talat et al (2000)* on 4 breeds (Rhode Island Red, broiler, Fayoumi and cross birds) that the average of cholesterol contents are 62.20; 49.75; 58.44; and 41.80 mg/dl, respectively.

Conclusion

The broilers that given CAKTAF which consists of fermented solid coconut oil and tofu waste will have more tenderness meat, than the broilers only consumed diets without CAKTAF. It means that the *Aspergillus niger* has an effect to the tenderness in the meat that feed CAKTAF.From the observation and analysis performed, that the cholesterol content also decrease if the broiler was given CAKTAF. This result showed that by using a mixture of coconut pulp (VCO waste) and tofu fermented by *Aspergillus niger* (CAKTAF) up to 25% in broiler rations can decreased the cholesterol content in the meat.

Uticaj dodavanja fermentisane smeše kokosovog ulje i čvrstog otpada tofua na mekoću i holesterol mesa brojlera

H. A. W. Lengkey, F. R.wolayan, M. Najoan, P. Edianingsih, B. Bagau

Rezime

Studija uticaja fermentisane smeše kokosovo ulja i tofu otpada na mekoću i holesterol mesa brojlersa imala je za cilj da se utvrdi uticaj mešavine kokosovog ulje i tofu otpada koji fermentisan sa *Aspergillus niger* u obroku na mekoću i holesterol mesa brojlera. Postoje neki pokazatelji, kao što su mekoća i sadržaja holesterola. Mešavina čvrstog kokosovog ulja i čvrstog otpada tofua, fermentisana pomoću *Aspergillus niger* (CAKTAF) može da se koristi kao dodatak u ishrani pilića. CAKTAF ima visok sadržaj proteina i mali procenat masti, što poboljšava kvalitet mesa. U ovoj studiji korišćeno je 120 jednodenvnih Cobb pilića u ogledu

koji je trajao 5 nedelja. Obrok i voda za piće su bili *ad-libitum*. Ovaj eksperiment koristi kompletno slučajan dizajn (CRD) 6 x 4, i sastojao se od šest tretmana (stopa upotrebe CAKTAF od 0% (R-0), 5% (P-1), 10% (P-2), 15% (R-3), 20% (R-4) i 25% (R-5) u obroku, a svaki tretman je ponovljen četiri puta Statistički testovi izvodeni korišćenjem analize varijanse i razlike između tretmana efekata su ispitane korišćenje Duncan-ovog testa višestrukog opsega. Rezultati ukazuju da upotreba do 25% CAKTAF u obroku, povećava mekoću mesa i smanjuje sadržaj holesterola u mesu.

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EFFECT OF EXTRUSION AND MICROWAVE ROASTIN ON CHEMICAL COMPOSITION AND PROTEIN SOLUBILITY OF FLAXSEED INTENDED FOR BROILERS NUTRITON

N. Puvača[1], V. Stanaćev[1], D. Milić[2], V. Stanaćev[2], Z. Pavlovski[3], Z. Škrbić[3]

[1]University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Republic of Serbia.
[2]Perutnina Ptuj-Topiko ad., Petefi Brigade 2, 24300 Bačka Topola, Republic of Serbia.
[3] Institute for Animal Husbandry, Belgrade-Zemun, Republic of Serbia.
Corresponding author: nikola.puvaca@stocarstvo.edu.rs
Original scientific paper

Abstract: Extrusion and micronisation are one of the most frequent heat treatments used for enriching nutritive quality of feed in Serbia. Aim of this study was to investigate effects of those heat treatments on basic chemical composition, crude protein solubility index (PDI) and urease activity in flaxseed. Flaxseed was extruded in an "Oprema-zootehnička oprema", type M2, model 1000 extruder at 125±1 °C and microwaved in a microwave oven SAMSUNG GE82N-B with LED display at 450W power for 3 and 5 minutes. Application of the extrusion led to statistically significant ($P<0.01$) urease activity reduction by 66.67%. Crude protein solubility index was 14.72% which is statistically highly significant ($P<0.01$) compared to other experimental treatments. Based on the gained results it can be concluded that dry extrusion can be a good choice for thermal treatment of flaxseed intended for usage in broilers chicken nutrition.

Key words: flaxseed, extrusion, microwave roasting, nutrition, broilers

Introduction

Flaxseed (*Linum usitatissiumum* L.) also known as linseed, is thought to be one of the world's oldest cultivated crops with evidence of cultivation dating back thousands of years. The crop is prized for its protein and oil content. The by-product remaining after oil extraction – flaxseed meal or linseed meal – is a source of protein used in animal nutrition. The seed is also used in animal production for its medicinal properties, in particular for its functions as a laxative as well as for improving skin and hair quality. Recently there has been a renewed interest in

using flaxseed and flax oil in animal diets as it can be used to alter the fatty acid composition of egg and meat products and, therefore, provide functional health benefits for the consumer *(Puvača et al., 2012a)*. Flaxseed has been consumed for centuries for its good flavor and for its nutritional properties. In recent years, as people have become more concerned about health, demand for flax in food and beverages, functional foods and dietary supplements has risen dramatically *(Vukelić et al., 2012)*. Flaxseed oil has a very healthy fatty acid profile, with low levels (approximately 9%) of saturated fat, moderate levels (18%) of monounsaturated fat and high concentrations (73%) of polyunsaturated fatty acids (PUFAs). The PUFA content comprises about 16% omega-6 fatty acids, primarily as linoleic acid (LA), and 57% alpha-linolenic acid (ALA C18:3n-3), an omega-3 fatty acid. Flaxseed is a rich source of protein and energy; however, the seed does contain some unique antinutritional components that need to be considered before inclusion of this feed in animal diet. Inclusion of flaxseed in animal diet, especially for chicken is very often accompanied with depressed growth, when it is used in amount higher than 5 or 10%. The presence of antinutritional factors and the physical structure are the main limiting factors. Cyanogenic glycosides, linatine, soluble nonstarch polysaccharides, trypsin inhibitor and urease are the main antinutritional factors in row flaxseed *(Shen et al., 2005a)*. Cyanogenic glycosides contain a cyanide group. Row flaxseed has these glycosides stored in the vacuole, but, if the plant is attacked, they are released and become activated by enzymes in the cytoplasm. These remove the sugar part of the molecule and release toxic hydrogen cyanide which is very toxic for the animals in small amount. Storing them in inactive forms in the cytoplasm prevents them from damaging the plant under normal conditions *(Ivanov et al., 2012)*. One of the most important antinutritive factors present in the raw flaxseed is trypsin inhibitor, which inhibits the effect of pancreatic proteases. Antinutritive thermo labile factors present in the flaxseed, including urease and trypsin inhibitors can induce growth inhibition, reduce feed efficiency utilization, goitrogens response, pancreas hypertrophy, hypoglycemia and liver damages of broiler chickens *(Beuković et al., 2010)*. Processing of seed improves nutrient utilization and can potentially reduce the negative impact of antinutritional components. Heat treatments like extrusion and microwave roasting, which are mutually different in their usage, types of heat source, construction of the equipment and applied parameters of the process, surely is one of the most significant alternatives for feed processing, which with its high nutritive values can satisfy high demands of modern nutrition *(Puvača et al., 2012b; Puvača et al., 2012c; Puvača and Stanaćev, 2012)*.

The aim of this study was to examine the effect of heat treatments such as extrusion and microwave roasting on chemical composition and reduction of urease activity in flaxseed, intended for usage in broiler chicken diets.

Materials and Methods

Experimental work considering chemical analyses and microwave roasting of samples was conducted at chemical laboratory for feed quality control at University of Novi Sad, Faculty of Agriculture at Department of Animal Science, while the extrusion process was conducted at Institute of Food Technology in Novi Sad. Experimental plan with flaxseed is given in Table 1.

Table1. Experimental plan with the flaxseed

Sample	Heat treatment			
	No treatment	Extrusion	Microwave roasting	Microwave roasting
Flaxseed	Row flax	125°C, 10 sec.	450W, 3 min.	450W, 5 min.

Samples of flaxseed cultivated in Vojvodina, Serbia in 2012 were used for the experiments. All treatments were done on flaxseed from the same producer and same batch. Samples were ground on hammer mill to pass 1 mm sieve and stored in dark at room temperature, after which chemical analysis were performed. Row flaxseed was used for extrusion. Flaxseed was extruded in an "Oprema-zootehnička oprema", type M2, model 1000 extruder (Ludberg, Croatia). Capacity of this extruder is 850-1000 kg/h extruded feed. Nominal power of electric motor is 75 kW, and of screw feeder with engine 1.5 kW. Working temperature measured in the head of extruder during extrusion of flaxseed was 125±1 °C, extruder capacity was 90%, current strength 85-90 A, and jet diameter 8 mm. Microwave oven SAMSUNG GE82N-B with LED display was used for thermal treatment of flaxseed samples. Each sample was put in a thin layer on glass plate with diameter of 15 cm, uniformly distributed and placed into the microwave oven. Operating frequency of microwave oven was 2450 MHz, and working power was 450W. Samples were treated for 3 and 5 minutes for that working power. For analyzing variations (analysis of variance – ANOVA) and Tukey's HSD post-hoc test for comparison of means of samples treated with the extrusion and microwaves of the same power, but for different time of heating was used STATISTICA SOFTWARE VERSION 12 (Statsoft, Tulsa, OK, USA).

Results and Discussion

Examinations of basic chemical composition and crude protein solubility index of row and heat treated flaxseed is given in Table 2.

Table 2. Chemical composition of row and heat treated flaxseed

	Row flaxseed		Extruded flaxseed		Microwaved flaxseed – 3min, 450W		Microwaved flaxseed – 5min, 450W	
%	χ	SD	χ	SD	χ	SD	χ	SD
Moister	5.82	0.06	5.93	0.03	3.31	0.01	1.71	0.01
Crude protein	23.26	0.49	23.69	0.21	23.57	0.39	23.99	0.07
Crude fat	39.52	0.34	38.82	0.41	39.22	0.20	38.42	0.23
Crude fiber	10.09	0.13	9.93	0.31	10.80	0.39	10.50	0.24
Ash	3.49	0.17	3.44	0.27	3.75	0.15	3.69	0.29
Ca	0.26	0.01	0.27	0.02	0.29	0.01	0.28	0.01
P	0.52	0.01	0.55	0.01	0.55	0.01	0.61	0.01
PDI	27.06^{a}	0.03	14.72^{b}	0.29	12.19^{c}	0.22	9.24^{d}	0.15

Results are presented as mean ± SD, n = 5,a-b, different superscripts within the same column indicate significant differences ($P \leq 0.05$)

While the obtained results of urease activity levels of flaxseed before and after microwave and extrusion treatments are given in Table 3.

Table 3. Flaxseed urease levels before and after heat treatments

	Row flaxseed		Extruded flaxseed		Microwaved flaxseed – 3min, 450W		Microwaved flaxseed – 5min, 450W	
	χ	SD	χ	SD	χ	SD	χ	SD
Urease activity, mgN/g/min	0.12^{d}	0.01	0.04^{a}	0.01	0.09^{c}	0.01	0.07^{b}	0.01
Index, %	100.00		33.33		75.00		58.33	

Results are presented as mean ± SD, n = 5
a-b, different superscripts within the same column indicate significant differences ($P \leq 0.05$)

Gained results shows that the application of extrusion process led to highly statistically significant ($P<0.01$) reduction of urease activity compared to untreated and microwave treated flaxseed. Reduction of urease activity of flaxseed roasted in duration of 5 minutes on 450W was statistically significant ($P<0.05$) higher compared to flaxseed roasted on 450W for 3 minutes. Reduction percentage of urease activity of applied heat treatments was 66.67; 25.00; and 41.67%, respectively. When it comes to crude protein solubility index (PDI), it can be noticed that extrusion process treatment had significant higher ($P<0.01$) values compared to the other experimental treatments. Recorded PDI values was 27.06; 14.72; 12.19; and 9.24% ($P<0.01$), respectively. *Shen et al. (2005b)* examined the effects of processing including pelleting, autoclaving, and microwave roasting on nutrient utilization in leghorn roosters. Pelleting the seed three times increased fatty acid retention by 29%. Microwaving for four minutes increased fatty acid

utilization by 39% and autoclaving increased fat utilization by 20%, demonstrating the positive effects of heat treatment on flaxseed utilization. Nitrogen retention was also significantly improved by heat and physical processing. *Feng et al. (2003)* concluded in their work that microwave roasting achieved the highest level of HCN reduction in linseed among autoclaving, pelleting and dry-heating in oven. They also showed that there were no major changes in the main nutrient and fatty acid profile caused by microwave treatment. *Wu et al. (2008)* investigated detoxification of linseed by extrusion. According to their results, extrusion can be considered a highly effective process in removing cyanogenic glycosides and trypsin inhibitors, but there was no data about comparison between extrusion and other processes. Similar results with extruded rapeseed grain gained *Stanaćev et al. (2011)* in their investigation. *Ivanov et al. (2012)* investigate effect of microwave heat treatment on the content of hydrogen cyanide, and consequently cyanogenic glycosides in flaxseed and came to conclusion that conditions of 400W of microwave power and 4 min 50 s of treatment were optimal for the reduction of HCN content under allowed limits. *Shen et al. (2005a)* fed broiler chickens 12% flax from 1-21 days and 15% from 22-40 days. Feeding whole flax reduced body weight, feed intake and feed efficiency but feeding flaxseed that had been previously pelleted and mashed significantly improved body weight gain, feed conversion efficiency and feed intake.

Conclusion

Based on the gained results it can be concluded that dry extrusion led to a significant inactivation of urease activity by 66.67% and crude protein solubility index was 14.72%. This finding makes an extrusion a good choice for thermal treatment of flaxseed intended for usage in broilers chicken nutrition.

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Efekat eksturiranja i mikrotalasnog prženja na hemijski sastav i rastvorljivost proteina zrna lana namenjenog za ishranu brojlerskih pilića

N. Puvača, V. S. Stanaćev, D. Milić, V. Ž. Stanaćev, Z. Pavlovski, Z. Škrbić

Rezime

Ekstrudiranje i mikronizacija predstavljaju najčešće upotrebljavane toplotne metode za poboljšanje hranljive vrednosti hraniva u Srbiji. Cilj ovog rada je bio da prikaže uticaj primenjenih toplotnih tretmana na osnovni hemijski sastav, rastvorljivost sirovih proteina (PDI) i aktivnost urease u zrnu lana. Ekstrudiranje lana obavljeno je na ekstruderu tip M2, model 1000 "Oprema - zootehnička oprema" pri radnoj temperaturi od 125±1 °C, dok je postupak mikrotalasnog prženja obavljen u mikrotalasnoj pećnici SAMSUNG GE82N-B sa LED displejom pri radnoj snazi od 450W tokom 3 i 5 minuta. Ekstrudiranje je dovelo do statistički značajne ($P<0,01$) redukcije aktivnosti urease (66,67%). Index rastvorljivosti sirovih proteina (14,72%) je bio statistički značajno veći ($P<0,01$) na tretmanu ekstrudiranja u poređenju sa ostalim tretmanima. Na osnovu dobijenih rezultata može se zaključiti da ekstrudiranje predstavlja dobar izbor kada su u pitanju toplotni tretmani za poboljšanje hranljive vrednosti lana, namenjenog za ishranu brojlerskih pilića.

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POSTER SECTION I

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EFFECTS OF DIET PHYSICALLY EFFECTIVE FIBER CONTENT ON FEEDING EFFICIENCY AND MILK PRODUCTION OF DAIRY COWS

B. Stojanović, G. Grubić, N. Đorđević, A. Božičković, V. Davidović, A. Ivetić

University in Belgrade Faculty of Agriculture, Nemanjina 6, 11081 Belgrade-Zemun, Republic of Serbia
Corresponding author: Bojan Stojanović, e-mail: arcturas@agrif.bg.ac.rs
Review paper

Abstract: Adequate long fiber, in a form that is physically effective, is necessary in total mixed ration for dairy cow to maintain proper rumen function promoting chewing and salivary buffer secretion and elevating rumen pH. The high correlations between chop length of forages - corn silage, alfalfa silage, oat silage and mean particle size of total mixed rations were determined. This indicates the significance of optimization of physical form of forages during ensiling to achieve the desired physical effectiveness of diet. The physical effectiveness of dietary particles can affect feed intake, rumen fermentation, digestive efficiency, milk production and composition. Milk yield is less sensitive to the effects of dietary peNDF (physically effective NDF). Low milk fat content is consistent with low mean rumen pH and low ratio of acetate to propionate. Milk protein content tended to be lower with reduced ruminal digestion and microbial synthesis.

Key words: forage chop length, diet particle size, lactating cows, nutrition

Introduction

Diets for lactating dairy cows low in fiber and high in starch are fed to increase intake of energy, but these diets increase the risk of ruminal acidosis. Time spent chewing, ruminal pH and milk fat percentage response, have been used to determine the effectiveness of fiber in diets for dairy cows (*Stojanović et al.., 2008*). Physically effective fiber (peNDF) is defined as dietary fiber which effectively stimulates total chewing activity (total time of eating and ruminating) and salivation as also affects on two-phase nature of ruminal content - larger floating particles-ruminal mat and liquid content that includes smaller feed particles, and is associated with the physically characteristics of the diet, primarily particle size (*Stojanović et al., 2010a*). Diets low in effective fiber and high in

fermentable carbohydrates can affect ruminal fermentation negatively. Fiber digestion is greatly depressed when pH declines below 6.0, the optimal ruminal pH for fiber digestion is around 6.5. A decrease in fiber digestion because of low ruminal pH can decrease ruminal digestion of the diet and, therefore, negatively affect production (*Stojanović et al., 2012a*). Short forage particle lengths can result in a reduction in chewing, saliva production, and rumen buffering, which increase the risk of subacute ruminal acidosis (*Stojanović et al., 2011a*). Minimal content of effective fiber is necessary in diet for dairy cows. Rations with adequate concentration of NDF but with fine chopped forages as source, causes the same metabolic disorders as at NDF insufficient diets fed dairy cows (*Stojanović et al., 2009b*). Dietary peNDF concentration depends on NDF content, mean particle size and forage cut length (*Stojanović et al., 2011b*). Effect of mean particle size of total mixed ration for lactating cows on milk fat percentage occurs with dietary NDF concentration under minimal requirements (25% NDF and 19% forage NDF in DM, *Stojanović at al., 2010b*). *Fox et al.* (*2003*) indicate that diets containing less than 20% NDF from forage reduce rumen microbial yield, and suggested that a reasonable minimum value for peNDF in dairy rations is 21-23% in DM. Microbial yield is reduced 2.5% for each percentage unit reduction in peNDF below 20 percent.

Mertens (*1997*) reports that required concentration of peNDF in diets for early- and mid-lactating cows is 20% DM for maintaining milk fat percentage of 3.4%. Concentration of peNDF on diet DM basis of 22% is necessary for providing average ruminal pH 6.

Earlier studies have shown that reducing the forage particle length can increase DMI (*Kononoff and Heinrichs, 2003*), have no affect on DMI (*Stojanović et al., 2012b*), or reduce DMI (*Krause and Combs, 2003*). Reducing the particle lengths of alfalfa silage and corn silage can lower milk fat (*Stojanović et al., 2012b; Krause and Combs, 2003*). However, other studies did not observe this effect (*Krause et al., 2002; Kononoff and Heinrichs, 2003*). The disparities among studies may be caused by interactions between numerous factors, including reduced forage particle size, grain source, forage source, forage to concentration ratio, range of particle lengths, and forage NDF content.

The objectives of this study were to consider the effects of forage particle size and the level of peNDF in the diet on feeding efficiency and milk production of dairy cows.

Effect of physical effectiveness of diet on production parameters of dairy cows

The high correlations between cut length of corn silage and alfalfa haylage and average particle size of diet were determined (r=0.97 and r=0.94, respectively),

(*Stojanović et al., 2009a; Stojanović et al., 2011c*). This indicates the significance of optimization of physical form of forages during ensiling to achieve the desired physical effectiveness of ration.

Kononoff and Heinrichs (*2003*) obtained that reducing cut length of alfalfa haylage (theoretical cut length of 22.3 mm, 2/3 with cut length of 22.3 mm and 1/3 with 4.8 mm, 2/3 with cut length of 4.8 mm and 1/3 with 22.3 mm, and 4.8 mm) in chemically identical diets (50% alfalfa haylage and 50% concentrate in DM basis) for cows in early lactation, increased DM intake linearly (20.8, 20.9, 22.0, 23.3 kg), increased digestibility of DM (63.1 and 66.5%), CP (53.2 and 58.6%) and NDF (44.7 and 48.1%). As a result of differences in digestibility, dietary content of NE_L tended to increase linearly from 6.15 to 6.57 MJ/kg DM. Milk production and percentage fat did not differ across treatments averaging 35.5 kg milk and 3.32% fat, while effect was observed for milk protein percent (2.82, 2.93, 2.91 and 2.90%, respectively for treatments). An effect was observed for mean rumen pH (6.09, 6.13, 6.15, 6.04) and acetate to propionate ratio decreased linearly (2.92, 2.88, 2.86 and 2.75) with decreasing particle size, as also total time chewing decreased (776.7, 768.3, 758.9 and 723.4 min/day).

In study of *Bhandari et al.* (*2008*) reducing the chop length of alfalfa silage (19 and 6 mm) did not affect DMI ($peNDF_{1.18mm}$ in diet 30.1 and 29.1%), but reducing the chop length of oat silage increased DMI ($peNDF_{1.18mm}$ in diet 30.2 and 28.9%) from 19.4 to 21.2 kg/d in mid-lactation Holstein cows fed diets containing 24% alfalfa silage and 24% oat silage (DM basis). Cows sorted against feed particles that were longer than 19 mm and sorted in favor of feed particles shorter than 8 mm, this sorting was greater for the diets containing the longer chop silages. Times spent eating, ruminating, chewing, and chewing per kilogram of DM were not affected by forage chop length and averaged 361, 575, 936 min/day, and 49.7 min/kg of DM, respectively. The chop lengths of alfalfa silage and oat silage did not affect the average daily rumen pH (6.24), the durations of time during which rumen pH was below pH 6 (450.3 min/day) and below pH 5.6 (143.8 min/day). Particle size of forages did not affect milk yield (36.1 kg/day), milk fat percentage (3.0%) or milk protein percentage (3.2%).

Bhandari et al. (*2007*) found that reducing the corn silage chop length (19 and 10 mm) increased dry matter intake from 22.3 to 23.2 kg/d ($peNDF_{8mm}$ in diet 18.7 and 17.3%), where reducing alfalfa silage chop length did not affect DMI ($peNDF_{8mm}$ in diet 18.7 and 17.3%), in mid-lactation Holstein cows fed diets containing 21.7% corn silage and 21.7% alfalfa silage (DM basis). The chop length of alfalfa silage did not affect rumen pH, whereas reducing the chop length of corn silage increased the rumen pH (6.12 and 6.20). Because cows selected against long feed particles, the peNDF content of the ingested feed was lower than the dietary content of peNDF, as a result, the intake of forage NDF might have been

insufficient to provide sufficient rumen buffering, resulting in SARA. The chop length of forages did not affect milk yield (38.2 kg/day), the percentages of milk fat (2.6%) and milk protein (3.3%), or milk fat and milk protein yield. Low milk fat percentage is regarded as a sign of subacute ruminal acidosis (SARA), although the rumen pH obtained was not indicative of SARA, the timing of rumen fluid collection may not have coincided with the lowest rumen pH. The low acetate to propionate ratio (2.3) also indicates on SARA occurrence.

Increasing mean particle length of oat silage (4.35, 4.46, 5.19 and 6.68 mm) in diets containing 25% corn silage and 25% oat silage (DM basis) for lactating Holstein cows, linearly decreased dry matter intake (from 22.1 to 20.4 kg/day), milk production (from 39.6 to 38.2 kg/day), and milk protein and yield (from 1074 to 1021 g/day) without affecting milk fat percentage (3.33%), milk fat yield (1295.8 g/day), increased chewing time (from 758 to 817 min/day), ruminal pH (from 6.11 to 6.27) and decreased ruminal volatile fatty acid concentration (from 106.7 to 100.3 mM), (*Leonardi et al., 2005*).

Table 1. Effects of reducing forage cut length on intake and productive performances of cows in early lactation (*Stojanović et al., 2012b*).

Item	Treatment			
	Long	Medium-long	Medium-short	Short
peNDF$_{8mm}$, %DM	20.15	18.91	18.60	14.57
DM Intake, kg/day	22.06	22.34	22.17	22.37
Digestibility, %				
DM	70.54	68.53	71.67	67.98
CP	73.60	71.56	77.90	76.41
NDF	53.90	54.40	55.01	58.66
Nonfiber carbohydrate (NFC)	91.99	89.18	86.80	87.68
Actual milk, kg/day	35.62	35.64	36.23	38.36
4% fat corrected milk (FCM), kg/day	32.82	31.39	31.22	34.71
Milk fat, %	3.50	3.21	3.10	3.35
Milk fat, kg/day	1.25	1.14	1.12	1.28
Milk protein, %	3.11	3.03	3.00	2.99
Milk protein, kg/day	1.11	1.08	1.09	1.15
Milk fat : protein ratio	1.13	1.06	1.03	1.12
DMI kg/kg milk	0.64	0.64	0.62	0.60
DM Intake kg/kg 4% FCM	0.70	0.73	0.73	0.68

Decreasing of corn silage and alfalfa haylage cut length (chop length 22, 19, 16 and 8 mm) and peNDF$_{8mm}$ concentration (from 20.15 to 14.57% in DM) in diet for cows in early lactation (57% of concentrate and 43% of forages, DM basis) did not affect DMI, increased the total tract digestibility of NDF (from 53.9 to 58.66%), and crude protein 71.56 to 77.90%), with the decrease in nonfiber

carbohydrate digestibility (from 91.99 to 86.80%). The increase in milk yield (from 35.62 to 38.36 kg), and decrease in milk fat (from 3.50 to 3.10%) and protein content (from 3.11 to 2.99%) were observed with the reduction of particle size in forages. There was no effect on milk fat daily yield, but the increase of milk protein yield (from 1.08 to 1.15 kg) was determined with the reduced forage cut lengths. Milk fat to protein ratio tended to decrease with the reduced forage cutting length. Decrease in forage particle size improved feed conversion ratio for milk production, improved digestibility and milk yield, while milk protein content was reduced (*Stojanović et al., 2012b*).

Krause et al. (*2002*) found that decreasing of mean particle size of total mixed ration (6.0 and 3.0 mm) based on alfalfa silage (forage to concentrate ratio 40:60), for lactating Holstein cows (60 days in milk), tended to decrease DMI (24.7 and 24.1 kg/day), increased digestibility of DM (72.2 and 74.2%), NDF (47.7 and 50.3%) and starch (94.4 and 96.1 %), decreased chewing time (from 761.5 to 553 min/day) and ruminal pH (6.02 and 5.81) while increased time below pH 5.8 (from 6.4 to 11.8 h/day), decreased milk fat content (3.61 and 3.45%).

According to *Krause and Combs* (*2003*), decreasing mean particle size of alfalfa (5.3 and 2.7 mm) and corn silage (5.6 and 2.8 mm) in total mixed rations (19.5 alfalfa and 19.5% corn silage, in DM basis) for cows at early lactation, decreased DMI (22.7 vs. 21.1 kg/day) and digestibility of DM (68.0 and 66.3%), as also chewing time (from 703 to 568 min/day) and increased milk yield (41.7 and 42.1 kg/day), did not affect on mean ruminal pH and time below pH 5.8 per day, decreased acetate to propionate ratio (2.28 and 2.07), milk fat percentage (from 2.95 to 2.66%) and milk fat yield (1.22 and 1.12 kg/day).

Conclusion

Effects of forage chop length and physically effectiveness of ration for lactating dairy cows on DM intake, digestibility and milk production is dependant of ration composition, forage to concentrate ratio, highly fermentable carbohydrate concentration and type of forages that are sources of physically effective fiber. The study demonstrated the need to accurately quantify the physical effectiveness of forages and diets. Using adequate chop length of silages and providing adequate mean particle length of total mixed rations, provides higher DMI, optimal ruminal pH and fermentation, higher digestibility, optimal milk composition and milk yield. Inadequate physically effectiveness of forages and diets, decreases feed efficiency and production of lactating cows, causing acidosis and connected metabolic disorders.

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Efekat sadržaja fizički efektivnih vlakana u obroku za mlečne krave na efikasnost ishrane i proizvodnju mleka

B. Stojanović, G. Grubić, N. Đorđević, A. Božičković, V. Davidović, A. Ivetić

Rezime

U kompletno mešanom obroku za krave u laktaciji, neophodan je adekvatan sadržaj vlakana odgovarajuće dužine, odnosno u formi koja je fizički efektivna, u cilju obezbeđenja normalnog funkcionisanja rumena, stimulisanja žvakanja i lučenja pljuvačnog pufera, kao i održavanja optimalne pH vrednosti. Utvrđeno je postojanje visoke korelacije između dužine odsečaka kabastih hraniva – silaže kukuruza, senaže lucerke, senaže ovsa, i prosečne usitnjenosti - veličine čestica kompletno mešanog obroka. Navedeno ukazuje na značaj optimizacije fizičke forme kabastih hraniva tokom siliranja, u cilju obezbeđenja potrebne fizičke efektivnosti obroka. Fizička efektivnost obroka utiče na konzumiranje hrane, ruminalnu fermentaciju, efikasnost varenja, proizvodnju i sastav mleka. Dnevni prinos mleka je manje osetljiv na sadržaj fizički efektivnih vlakana (peNDF). Smanjenje sadržaja mlečne masti se podudara sa nižom ruminalnom pH vrednošću, i užim odnosom molarne koncentracije propionata i acetata. Sadržaj proteina u mleku pokazuje tendenciju smanjenja sa smanjenjem ruminalne svarljivosti i mikrobijelne sinteze.

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CHARACTERISTICS OF FEEDING BEHAVIOUR OF DAIRY COWS DURING EARLY LACTATION

D. Kučević[1]*, S. Trivunović[1], M. Plavšić[1], M. Radinović[1], V. Bogdanović[2]

[1]University of Novi Sad, Faculty of Agriculture, Department of Animal Science, Novi Sad, 21000
[2]University of Belgrade, Faculty of Agriculture, Zootechnical Institute, Belgrade – Zemun, 11080
Corresponding author:
*Denis Kučević; e-mail: denis.kucevic@stocarstvo.edu.rs
Original scientific paper

Abstract: The study is aimed to determine certain features of feeding behavior of dairy cows. The investigation was based on data of 60 high-yielding dairy Holstein-Friesian cows (red and black) with yield 7.800 kg per lactation. The study was conducted from the 7th to the 90th day of lactation. Duration of every single visit at feeders for each individual cow has been recorded by using feeder acquisition system (single feed place) and electronic identification of animals with programmable gate for the access control. System was equipped with infrared sensors for recording the time of entry and the time they leave. The results showed that characteristics of feeding behavior such as number of visits per day/cow, meal length per day/cow and total feeding duration per day/cow amounted (mean ± SD): 20.8 ±6.55 visits, 6.3□± 2.14 minutes, 215.8□± 39.2 minutes.

Key words: Dairy cow, feeding behavior, early lactation

Introduction

Feeding behaviour of lactating cows cannot be defined through a single pattern because the expression of animal's activity during feeding is affected by a large number of various factors (rearing system, group size, available space, diseases, quality and taste of meals, individual animal characteristics, etc.). *Ivetić et al. (2009)* concluded that feeding behavior is different in the different system of rearing at the same breed and lactation stage of dairy cows.

Intensive milk production requires a proper norming of meal regarding to the undegradable and total proteins, as well as providing sufficient quantity of energy, minerals, vitamins, additives, etc. *(Sretenović et al., 2007).*

The most sensitive and physiologically stressful period during the annual cycles of dairy cows is period before and after calving i.e. during early lactation. It

is well known that the maximum feed intake during lactation does not follow the highest milk production, which represents excessive burden for high yielding dairy cows. One of the most important activities during the feeding behavior of dairy cows is the time required for feed intake (at feeder), which has direct influence on the amount of feed intake *(Grant, RJ and JL Albright, 2001)*.

Melin et al. (2005) reported that the change of meal composition, feeding techniques etc., can significantly influence feeding behaviour of dairy cows (frequency of visits at feeder, meal length, amount of food intake, etc.). Besides the rearing system and breeding conditions, *Urton et al. (2005)* emphasize the importance of disease on change in behavior of dairy cows during feeding. Metritis diseased cows stay at feeder on average 22 minutes shorter i.e. reduce the time of feeding.

Literature sources noticed lots of differing results in relation to certain forms of feeding behavior of lactating cows, for example, within 24 hours total feeding duration lasts on average from 3 to 6 hours per cow and approximately occurs from 9 to 30 visits at feeder. The reason for these results lies precisely on the fact that many factors affect feeding behavior of cows as well as insufficiently defined methods. The study of feeding behavior in the specific rearing conditions provides useful information that can be used for adjustment of breeding technology in accordance with the animals needs.

Materials and Methods

The investigation was based on data of 60 high-yielding dairy Holstein-Friesian cows (red and black) with yield 7.800kg per lactation. Cows were kept in free system of rearing and fed by using automatic feed system (feeder). The number of animals and feed places was in relation 2:1. Dairy cows were fed once per day in the form of complete mixed ration without restrictions (ad libitum). The study was conducted from the 7th to the 90th day of lactation. Individual control of access to feeder for every single animal was monitored by using electronic animal identification equipped with infrared sensors as well as programmable gate for the access control. Based on that, it was possible to determine every single visit of each individual cow i.e. the number of visits at feeder per day and cow. Regarding the time of entry and the time of leaving were defined meal length and total feeding duration.

Data was analysed by using the software Statistics 10 *(stat. Soft. Inc. 2012)*. General variability of observed traits was analysed by using the descriptive statistical analysis.

Results and Discussion

During the research period from the 7th to the 90th day of lactation, the average meal length per day/cow amounted 6.3□± 2.14 minutes, number of visits per day/cow was 20.8 ±6.55 and total feeding duration per day/cow was 215.8□± 39.2 minutes (table 1).

Table 1. Observed characteristics of feeding behavior

Parameters	X	SD	min	max
Number of visits[1]	20.8	6.55	6	48
Meal length[2] (min./visit)	6.3	2.14	1,89	22
Total feeding duration[3] (min./day)	215.8	39.2	65	450

[1]Number of all visits at feeders (with and without food intake) per day and cow
[2]Time difference between start and end of visits at feeder
[3]Total feeding duration per day and cow

Notable fluctuations in relation to total feeding duration were recorded during the period of research as well as permanent increase of total time of rentention at feeder. An increase from 180 min. to 220 min. was registered in the first 5 weeks, while the time was prolonged for 10 min. on average to the end of the 90th day (Figure 1).

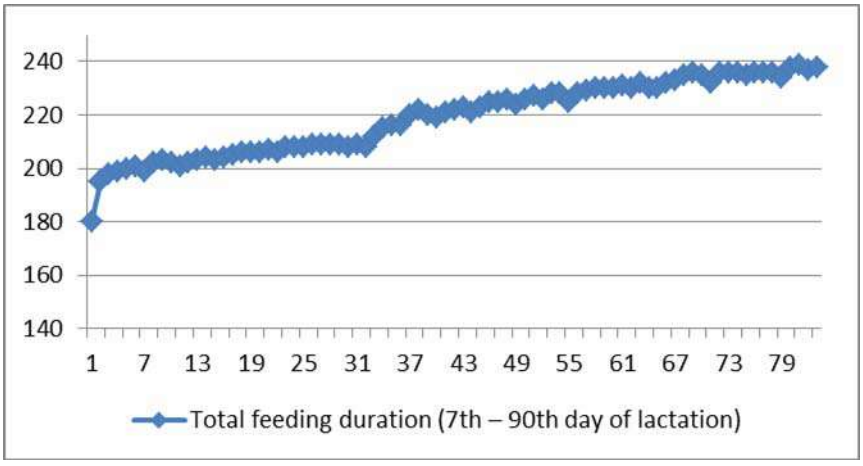

Figure 1. Total feeding duration per cow and day from 7th to 90th day of lactation

In our study the total feeding duration amounted 215.8 minutes and this result is similar to *Shabi et al. (2005)* and *Kaufmann et al. (2007)*. Slightly longer time of retention at feeder (253 min.) is reported by *Vasilatos and Wangsness (1980*) as well as by *Dado and Allen (1994)* - 301 min / day. One of the reason for the various feeding times during the first 3 months of lactation, *Bencsik et al. (2004)* noted as considerable factors cows age and parity (primi – and multiparous).

If it is observed the period from the beginning of lactation (early lactation) to the middle of lactation, most researchers report about permanent increase of total time of retention at feeder. *Tolkamp et al. (2000)* stated that in the period from early lactation to middle lactation, total feeding duration per day/cow increasing and reaches an average value 225.1 min. *DeVries et al. (2003)* also confirms that increase of total feeding duration is particularly noticeable since the beginning of lactation until the 15th week.

Average meal length per day/cow amounted 6.3□± 2.14 minutes. At the beginning of lactation meal length was slightly shorter (about 5.9 min.) and stretched evenly until the 5th week of lactation. Meal length exceeded 7 min. only in last third of the study period (Figure 2).

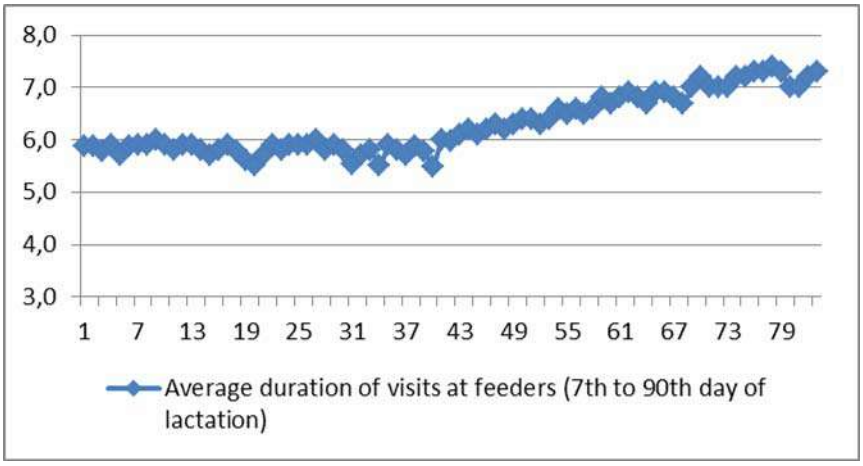

Figure 2. Average duration of visits at feeders per cow and day from 7th to 90th day of lactation

Determined average meal length per day/cow in our research was in accordance with the results by *Kaufmann et al. (2007)*, 6.79 min / visit and *Tolkamp et al. (2000),* 6.8 min / visit. However, *Vasilatos and Wangsness (1980)*

stated that the average meal length per day/cow had much higher values (20.9 min / visit).

An average number of visits at feeder during the day per cow was 20.8. This result was vastly higher than results of the other researchers. *Shabi et al. (2005)* reported that the average number of visits was 12 per cow, while *Tolkamp et al. (2000)* and *DeVries et al. (2003)* reported that the number of visits was only 6 to 7 per cow. *Kaufmann et al. (2007) noted* that average number of visits were vastly higher than other results (32.07 visits).

During the first week an average number of visits per cow was around 19, but in the last weeks achieved almost 22 visits / cow / day (Figure 3).

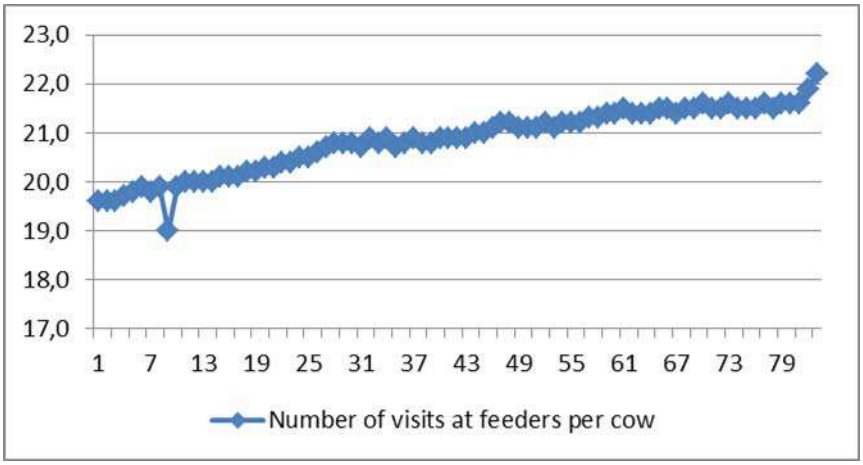

Figure 3. Number of visits at feeders per cow and day from 7th to 90th day of lactation

These excessive oscillations in determined number of visits can be explained by variety methods of measurement. In fact, in our research, every time the animal pull out head back over the feeder and put its head back again (although not completely left the feed place), there was registered a one visit. This method of recording (measurement) significantly contributed to the higher average number of visits.

Dado and Allen (1994) reported that existing differences in the parameters of feeding behavior of cows come from the differences in the levels of production of experimental animals, the chemical composition and physical structure of feeds, environmental factors (including farm management) as well as various definitions of examined parameters and methods of measurement.

Conclusion

The study of feeding behavior of cows in early lactation from the 7th to the 90th day included the following parameters: total feeding duration, average meal length per day/cow as well as an average number of visits per cow. Determined number of visits during the day was not in accordance to the results of the other authors, while other parameters were consistent with published results.

By the analysis of feeding behavior of dairy cows, results can be used to adjust the breeding technology in accordance with the animals needs. It can be concluded that the parameters of feeding behavior in early lactation can affect through many different factors influencing the each individual animal. Therefore, it is necessary to conduct research in concrete conditions of production for each individually dairy farm in order to identify all relevant factors that may affect feeding behavior as well as precisely define the methods for measuring and testing parameters.

Karakteristike hranidbenog ponašanja krava u ranoj laktaciji

D. Kučević, M. Plavšić, S. Trivunović, M. Radinović, V. Bogdanović

Rezime

Cilj istraživanja je bio da se utvrde pojedine karakteristike hranidbenog ponašanja krava u ranoj laktaciji. Za potrebe istraživanja su korišćeni podaci za 60 krava muzara holštajn frizijske rase (crveni i crni), prosečne mlečnosti 7 800 kg u laktaciji. Istraživanje je sprovedeno u periodu od 7. do 90. dana laktacije. Merenje trajanja svake posete jaslama za svaku pojedinačnu kravu je utvrđeno uz pomoć automatskog sistema za ishranu, elektronskog sistema za identifikaciju životinja kao i programiranih ulaznih vrata za kontrolu pristupa na jaslama. Sistem je bio opremljen infra crvenim senzorima za registraciju ulaza i izlaza krava sa jasala. Ispitivane karakteristike hrandbenog ponašanja su pokazale da je broj poseta po kravi i danu, prosečno zadržavanje (trajanje) posete kao i ukupno vreme provedeno na jaslama iznosili (X□± SD): 20,8 ±6.55 poseta/krava/dan, 6.3□± 2.14 minuta krava/dan i 215.8□± 39.2 minuta /krava/dan.

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SEASON'S INFLUENCE OF SOME ECOLOGICAL FACTORS ON MILK PRODUCTIVITY IN COWS

K. Krastev, S. Grigorova

Institute of Animal Science, Kostinbrod, Bulgaria
Corresponding author: svet.grigorova@abv.bg
Original scientific paper

Abstract: The season's influence of ecological factors (temperature, relative humidity and wind velocity in the barn) on the dynamics of milk production in clinically healthy cows at 305-days lactation length was investigated. A total of 87 cows from Bulgarian Black-and-White population were raised year-round tied in a barn, constructed of concrete steel panels with natural ventilation. The animals were divided into 4 groups, 16 cows each. The cows from different groups were calved as follow: first group – in December; second group – in March; third group – in June; fourth group – in September. Excrements were disposed by flat-chain transporter. Cows were milked twice a day by aggregates in milk-cans, watered in individual watering-troughs and fed in cement cribs. Animals were fed corn silage with 25-30% dry matter and concentrate feed. In summer silage was replaced with a green alfalfa. It was found that the biological regularity for decrease in milk productivity at the end of the lactation is directly influenced by the season, that is a total ecological factor. This regularity increases under the influence of low temperature and high humidity when lactation ends during autumn and winter. The opposite was observed in cows which the second half of lactation proceeds during spring and summer. In this case the biological regularity is suppressed and milk production stays or sinks under influence of high temperature and low humidity in the barn.

Key words: milk production, Black-and-White cows, season's influence, ecological factors

Introduction

Under normal situations milk production increases during the first six weeks of lactation and then gradually decreases. A number of ecological factors are known to exert influence on the milk yield of dairy animals. The effect of environmental factors on dairy production of cows has been well documented *(Baiwa et al., 2004; Chagunda et al., 1995; Javed et al., 2000; Kunaka and*

Makuza, 2005; M'hamdy et al., 2012). Some of them are the seasonal changes of ecological parameters in a production environment *(Krastev et al., 2000)*. The small scale farms which include cattle farms are unstable that is why the influence of the environmental factors is important for their existence *(Riclefs, 1993)*. Production environment is limiting factor for cow welfare and milk production *(Brouk et al., 2003). Zurbrigg et al. (2005)* considered that 30% of the cows' milk productivity depends on abiotic environmental factors like temperature, relative humidity and wind velocity. *Krastev and Gaydarska (2000)* established that the microclimate in the barn is important to maximize the milk production in Black-and-White cows.

High summer temperature and relative humidity induce temperature overload which exceeds body capacity for heat dissipation and ultimately results in stress *(Gudev, 2006)*. Cows respond to heat by reduction of feed intake *(Bernabucci et al., 1999; Ominski et al., 2002; Schutz et al., 2008)* and milk yield *(West et al., 2003)*.

Favorable ambient temperature for dairy cows is within 5-22°C. High productive animals are more sensitive to high environmental temperature. According to Berman (2005) the difference critical temperature increases by 5°C at any increase of milk yield by 10 l. *West (2003)* supposed that selection for heat tolerance could improve milk yield in dairy cattle under high ambient temperature. Therefore it is necessary to include heat tolerance as a selection criterion *(Ravagnolo and Misztral, 2000).*

The aim of our investigation was to examine season's influence of ecological factors like temperature, relative humidity and wind velocity in the barn on dynamics of milk productivity in clinically healthy cows from Bulgarian Black -and-White population at 305 days lactation length.

Materials and Methods

An experiment was conducted with 87 clinically healthy third lactation cows, Bulgarian Black- and -White population at 305 days lactation length. The animals were divided into 4 groups, 16 cows each. The cows from different groups were calved as follow: first group – in December; second group – in March; third group -in June; fourth group - in September.

Cows were raised year-round tied in a barn constructed of concrete steel panels with natural ventilation. Capacity of the building is for 100 dairy cows distributed into two rows of 50. Walls are made of single layer panels with thickness 0.18m and joints are restrained without plaster. Roof is flat and constructed of double T-shaped concrete steel panels over which is laid thermo and hydro isolation with a total thickness of 0.30m. There are windows with

dimensions 1.20/ 0.80m at 1.80m above floor level along the whole length of both longitudinal walls with a light coefficient 1:14. Cribs are placed opposite to each other with a feeding path between them. Beds were made of floor bricks with a 3˚ inclination. Excrements were disposed by flat-chain transporter. Water was supplied via individual automatic watering trough. Animals were fed corn silage with 25-30% dry matter and concentrate feed. In summer silage was replaced with a green alfalfa. Feeding of succulent and roughage was mechanized and this of concentrated fodder - manually. Cows were milked in the barn twice a day by aggregates in milk-cans.

Daily temperature (in °C), relative humidity (in %), wind velocity (in m/s) in the barn, as well as milk yield (in kg) were controlled. Temperature and relative humidity were measured by using a thermohygrograph TZ-18. Wind velocity in the barn was determined by Hill kata thermometer. Measuring equipment were located at the level of cows' living zone. Measurements were made at four opposite points of the barn throughout the experimental period. All date are presented as month averages. Statistical analysis of obtained results was determined by Excel 2000, Anova program. Daily milk productivity is submitted as means ± SEM.

Results and Discussion

The farm is situated in a moderate continental climate with four clearly defined seasons. This climate is characterized by cold, wet winters and hot, dry summers. Table 2 shows the average monthly values of investigated ecological parameters in the barn (temperature, relative humidity and wind velocity) and average monthly values of daily milk yield. Average monthly temperature in the barn during the experimental period varied between 3.6 °C and 31.2 °C. Absolute minimum value of temperature in this period was 2.9°C (in December) and absolute minimum reached 36.6 °C (in August). The average monthly relative humidity fluctuated between 41% and 91%. The absolute minimum value of this index was 36% (in August) and its maximum - 96% (in December). The average monthly wind velocity in the barn was within 0.30m/s to 0.72m/s. The minimum value of this parameter was measured in January (0.26m/s) and its maximum – in August.

Table 1. Average monthly parameters of environmental factors (temperature, relative humidity and wind velocity in the barn) and average monthly milk yield (mean ±SEM)

Season	**Month**	**Temperature, °C**			**Relative humidity, %**			**Wind velocity, m/s**			**Average milk yield, kg/cow/day mean** ±*SEM*	**Number of dairy cows**
		mean	min	max	mean	min	max	mean	min	max		
	January	3.6	3.0	8.8	88	84	93	0.30	0.26	0.44	10.24 ± 0.18	65
Winter	February	4.0	3.3	9.1	86	82	91	0.31	0.27	0.46	10.98 ± 0.16	65
	March	5.8	4.5	13.7	85	80	91	0.35	0.29	0.51	11.78 ± 0.14	87
	April	11.3	6.8	20.1	61	58	73	0.36	0.30	0.54	13.21 ± 0.11	66
Spring	May	14.2	7.7	21.8	74	57	88	0.38	0.33	0.59	15.07 ± 0.14	66
	June	21.9	11.7	29.8	60	49	76	0.47	0.39	0.67	16.87 ± 0.16	87
	July	24.9	14.0	31.2	58	42	81	0.62	0.44	0.78	16.30 ± 0.14	65
Summer	August	31.2	17.2	36.6	41	36	77	0.72	0.49	0.98	12.41 ± 0.17	65
	September	22.8	12.4	28.6	53	43	62	0.67	0.46	0.75	13.99 ± 0.15	87
	October	15.9	7.9	18.2	68	55	78	0.49	0.40	0.71	14.68 ±0.16	65
Autumn	November	8.5	4.4	12.5	87	74	95	0.40	0.39	0.60	13.88 ± 0.11	65
	December	4.3	2.9	12.1	91	77	96	0.35	0.30	0.50	11.72 ±0.16	87

Influence of seasons on the dynamics of cow's milk productivity is presented graphically in Figures 1-4. The curve of milk productivity in first group of cows, calved in December (Figure1) was maintained at the same level, with small fluctuations, up to March, followed by increase, reaching its highest point (16.87kg/day) in June. Then it decreased again to reach 13.99 kg/day at the end of lactation. The second group of cows, calved in March (Figure 2), when the weather was still cold (average monthly temperature 5.8 °C, relative humidity 85%, wind velocity 0.35m/s). Their daily milk yield at the onset of lactation was 11.78kg. Average daily milk yield in this group like that in first group increased sharply (16.78 kg/day) with the enhancement of ambient temperature in June (21.9 °C, relative humidity 60% and wind velocity 0.47m/s). Average daily milk yield

declined in August (12.41kg/day), then it increased in October (14.60 kg/day) and gradually declined until December (11.72 kg/day).

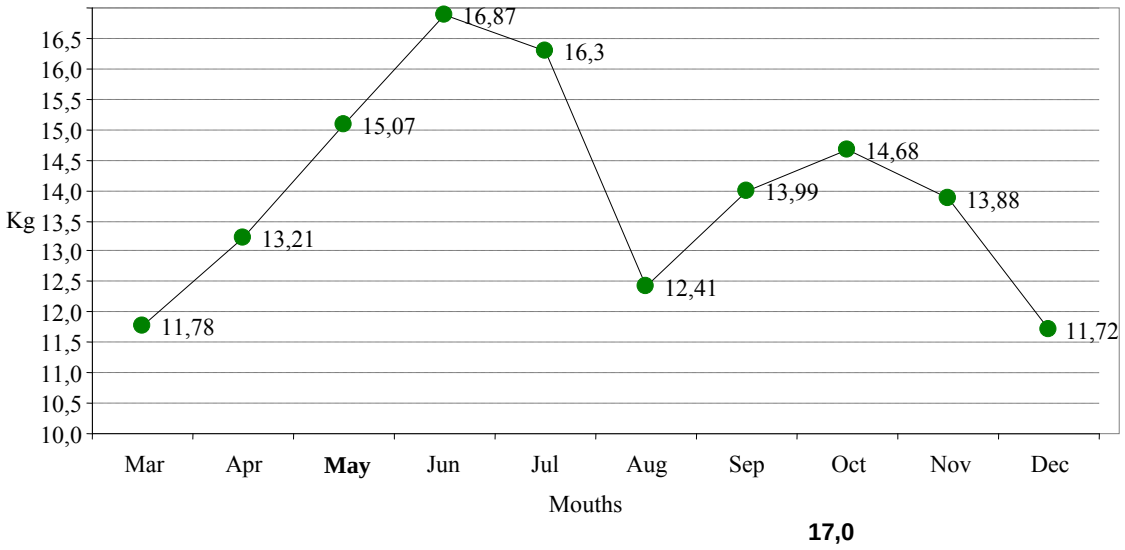

Figure 1. Milk productivity (in kg) of cows calved in winter particularly in December

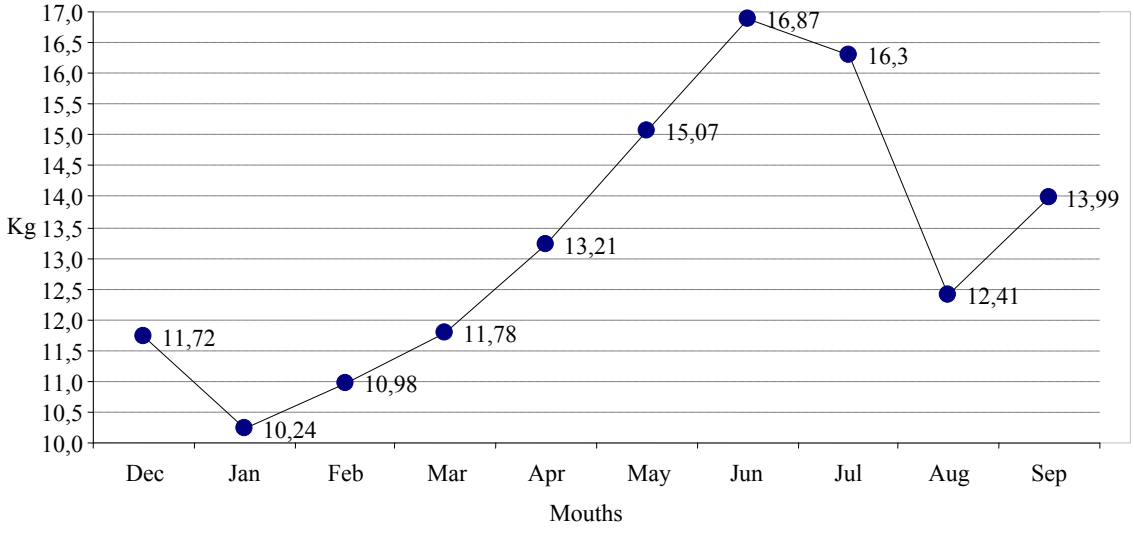

Figure 2. Milk productivity (in kg) of cows calved in spring particularly in March

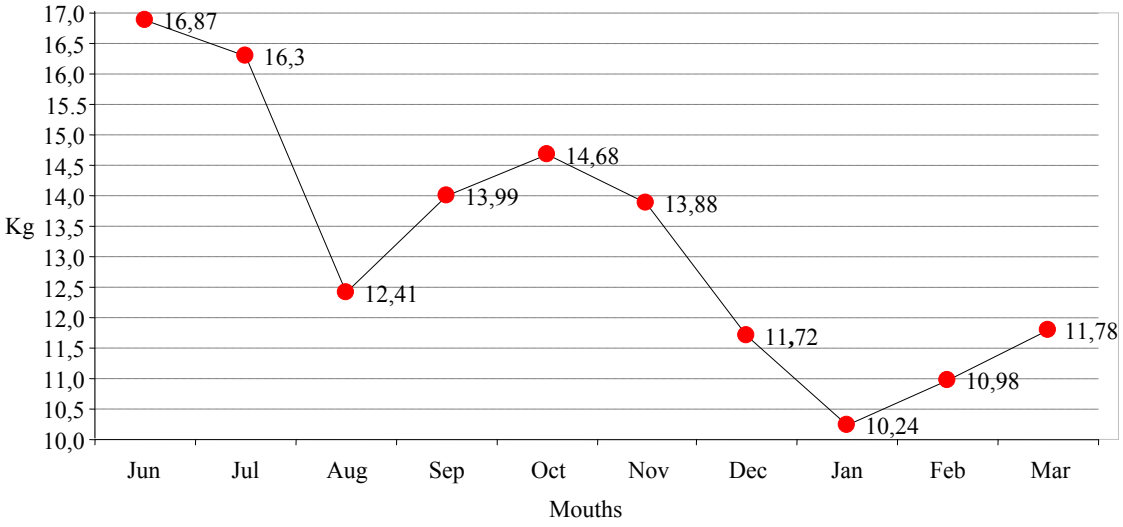

Figure 3. Milk productivity (in kg) of cows calved in the summer particularly in June

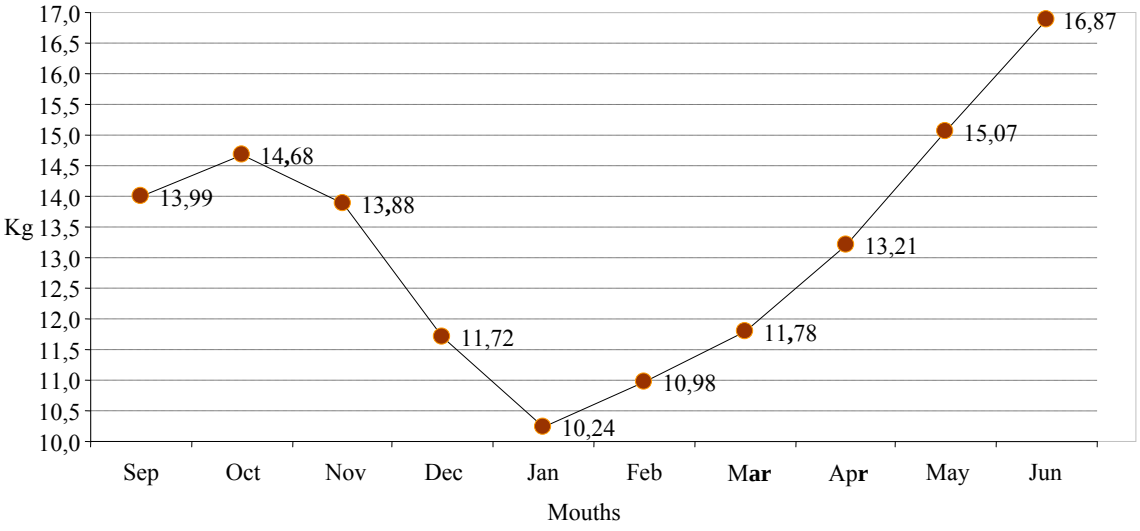

Figure 4. Milk productivity (in kg) of cows calved in autumn particularly in September

Unlike the first two groups the third group of cows, calved in June (Figure 3) produced 16.87l/day at the beginning of lactation. Average daily milk yield was lowest in August (12.41kg) when the average monthly temperature, relative humidity and wind velocity were 31.2 °C, 41% and 0.72m/s respectively, followed by a sharp increase without fluctuations, reaching its highest level in October (14.68kg). The fourth group of cows calved in September (Figure 4) had the lowest milk productivity in January (10.24kg), followed by a gradual increase until June (16.87kg).

The obtained in this investigation results indicate that milk yield of cows is affected by season of calving. Temperature, relative humidity and wind velocity in the barn, which are different in various seasons have a direct influence on the dynamics of dairy production. Our results are in agreement with the data of *Javed et al. (2004); Kunaka and Makuza (2005); M'hamdi et al. (2012)*, who studied the influence of season of calving on milk yield in Frisian, Holstein-Frisian and Tunisian Holstein cows, respectively.

Conclusion

It was found that the biological regularity for decrease in milk productivity at the end of the lactation is directly influenced by the season, that is a total ecological factor. This regularity increases under the influence of low temperature and high humidity when lactation ends during autumn and winter. The opposite was observed in cows which the second half of lactation proceeds during spring and summer. In this case the biological regularity is suppressed and milk production stays or sinks under influence of high temperature and low humidity in the barn.

Sezonski uticaj nekih ekoloških faktora na proizvodnju mleka krava

K. Krastev, S. Grigorova

Rezime

U ovom radu je ispitivan sezonski uticaj ekoloških faktora (temperatura, relativna vlažnost i protok vazduha u štali) na dinamiku proizvodnje mleka klinički zdravih krava, u laktaciji u trajanju od 305-dana. Ukupno 87 krava iz bugarske crno-bele populacije su gajena tokom cele godine u vezanom sistemu u štali, izgrađenoj od betonskih čeličnih panela sa prirodnom ventilacijom. Životinje su

podeljene u 4 grupe, 16 krava u svakoj grupi. Krave iz različitih grupa su se otelila na sledeći način: prva grupa - u decembru, druga grupa - u martu; treća grupa - u junu, četvrta grupa - u septembru. Krave se muzu dva puta dnevno mašinski u kontejnere za mleko, vodu dobijaju u pojedinačnim koritima a hranu u cementnim hranilicama. Životinje su hranjene silažom kukuruza sa 25-30% suve materije hrane i koncentrata. U leto silaža je zamenjena sa zelenom lucerkom. Utvrđeno je da je biološka zakonitost smanjenje proizvodnje mleka na kraju laktacije pod direktnim uticajem sezone, odn. ukupnog ekološkog faktora. Ova zakonitost povećava se pod uticajem niske temperature i visoke vlažnosti, kada se završava laktacija tokom jeseni i zime. Suprotno je primećeno u krava kod kojih je druga polovina laktacije tokom proleća i leta. U ovom slučaju biološka zakonitost je potisnuta i proizvodnja mleka ostaje ili opada pod uticajem visoke temperature i niske vlažnosti vazduha u štali.

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SAFETY OF GOLIJA MILK AND CHEESE FROM THE RADIATION-HYGIENIC ASPECT

M. Vićentijević, R. Mitrović, V. Vuković

Scientific Veterinary Institute of Serbia, Belgrade, NIVS-Belgrade Highway 3, N. Belgrade,
Corresponding author: vicamihajlo@yahoo.com
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Abstract: In the process of production, processing and treatment of milk, in addition to biological contamination (microorganisms), many abiotic substances can be contaminants. These substances are the product of human activities. They are introduced into milk from the environment, as a result of imperfect technology of milk production, and in many cases of inability and ignorance of stakeholders in dairy industry to prevent contamination. In regard to the hygiene of milk, special attention should be paid to the radioactive substance. The paper presents the results of the Laboratory for radiation hygiene - "LABRAH" at the Institute of Veterinary Medicine of Serbia in Belgrade. Radiation-hygienic control was carried out using gamma spectrometric analysis of the radiation products from milk and cheese. The results obtained clearly indicate that the production of milk and cheese is safe and within limits for the appropriate region climate.

Key words: Radiation-hygienic control of milk and cheese, LABRAH, ^{137}Cs, ^{40}K, ^{238}U

Introduction

In cases of incidence of radioactive fallout, as well as various types of atmospheric residues, grazing animals are especially endangered - milking cows (cows, sheep, goats, etc.), i.e. animals in whose nutrition it is necessary to use green mass. Due to the radioactive contamination of milk, from the medical and hygienic point of view, there is a wide range of problems caused by further treatment and processing of milk, since all the paths of the transfer of radionuclides end in human population, as the ultimate consumer of foodstuffs - milk and milk products. It is well known that the biologically significant radio nuclides ($^{89,90}Sr$, $^{134,137}Cs$, ^{131}I, ^{103,l06} Ru, etc.) after ingestion are very quickly eluted from the body of animals. For example, ^{131}I occurs in milk 3 to

6 hours after ingestion and similar situation is with the other radionuclides from a mixture of fission products. In this way, the residues of radioactive substances occur in milk and dairy products.

In order to fully understand the problem of risk to the dairy industry from radioactive agents, certain knowledge about radiation, radioactive contamination and biologically significant radionuclides is essential.

Biologically significant radionuclides in milk and dairy products

Biologically significant radionuclides (BSR) in milk production may be considered from the aspect of dose in human tissues. It can be concluded that the importance of radioactive contamination should be evaluated on the basis of radioactively contaminated milk and its effect on total dose in the human tissues. Attention should be paid to those radionuclides in milk, which will affect the incidence of the highest doses in the human tissues, which is why they can be called critical radionuclides in milk, which is classified as key foodstuffs.

Control of radioactive fallout on a global scale, studies on the grounds for nuclear weapons testing, and laboratory studies have suggested, and already it is absolutely accepted, that radioactive iodine (^{131}I) radioactive strontium (^{89}Sr i ^{90}Sr) and radioactive cesium (^{137}Cs) release the highest doses. Over the last decade, there has been significant progress in the development of countermeasures for prevention or reduction of contamination of animal products from radioisotopes of iodine, cesium and strontium *(Howard et al., 2001)*. Milk that originates from dairy cows that graze on pasture during the deposition of radioactive fallout, is extensively contaminated by these critical radionuclides, and they deserve special attention.

Radioactive strontium

Radioactive strontium is representative of fission products from the group of alkaline earth metals, with significant fission yield of about 5%. When it comes to radioactive strontium, usually two of its radionuclides are referred to (^{89}Sr and ^{90}Sr) which differ by some features (Table1).

Table 1. Characteristics: ^{89}Sr and ^{90}Sr

Radionuclides	Type of radiation	Maximum energy (MeV)	The half-life		
			Physical ($T_{½}$)	Biological ($B_{½}$)	Effective ($E_{½}$)
^{89}Sr	ß	0,58	55 d		50 d
^{90}Sr	ß	0,19	~ 28 g	10 g	~ 18 g

In regard to radiation emissions, both strontium radionuclides are "pure" beta emitters with low energy beta radiation - the so-called "soft" beta radiation - which is why these radionuclides have great biological significance. It is well known that the low energy beta radiation in interaction with matter produces more ion pairs compared with the high-energy beta radiation.

Both strontium radionuclides behave chemically identically, and therefore their metabolic behavior in the body is identical.

Main biological characteristics of both strontium radionuclides are contained in the following statements:

a) In the organism they behave analogously to calcium, which means that the ^{90}Sr ions are deposited in bone system in the form of hydroxyapatite. Accordingly, they are called. osteotropic radionuclides because the skeletal system represents their critical organ.

b) The bones of young organisms bind 5-10 times greater quantity ^{90}Sr than bones of adult organisms, because the body requirements in calcium during skeletal growth are large. This fact is of particular importance when it comes to feeding children, because if the food is lacking calcium, bone cells will readily bind radioactive strontium as osteocytes have no power of selective separation of strontium ions from calcium ions.

Previous studies have shown that substances that enhance absorption of Ca (milk, lactose, amino acids, lysine, arginine) increase partially also resorption of ^{90}Sr. Radioactive contamination of dairy cows, after one-time ingestion, induces a rapid increase in the level of activity of radioactive strontium in the blood and quick excretion through milk, so in approx. 30 hours the maximum concentration is reached. During the next 5 days levels of activity gradually decline. However, long-term daily intake of radioactive strontium with food results in increased levels of activity in milk, where after a period of 7-10 days the level of saturation is established, and activity of radioactive strontium in the milk of dairy cows is still maintained at a level of about 1-3 %, and in sheep and goats of about 4% of the ingested daily activities through food and water *(Fesenko et al., 2007).*

Radioactive cesium

Radioactive cesium is represented by two BSR - ^{134}Cs and ^{137}Cs - fission products with different fission yields. For example, while radionuclide ^{137}Cs has a high fission yield of 6.2%, radionuclide ^{134}Cs has a low fission yield of 0.01%, which results in their different representation of the biosphere. In addition, these two radionuclides differ from one another in some physical characteristics (Table 2).

Radioactive decay of ^{137}Cs is followed by beta emission of different energy, transforming into a radioactive isomer of barium - ^{137m}Ba - with very short half-life ($T_{1/2}$ = 2,7min), who as one show gamma quants - 0.66 MeV energy - becomes stable isotope ^{137}Ba.

Table 2. Characteristics: $^{134137}Cs$

Radionuclides	Type of radiation	Maximum energy (MeV)	The half-life	
			Physical ($T_{½}$)	Biological ($B_{½}$) both radionuclides
^{134}Cs	ß	0,16	2,3 g	20-70 d for cows 17 d for sheep 31 d for goat
	γ	1,6		
^{137}Cs	ß	0,51 (92%) 1,17 (8%)	30 g	
	γ	1,2		

In terms of chemical properties both cesium radionuclides behave identically, so their metabolic behavior in the animal body is identical.

Main biological characteristic of radioactive cesium is that cesium ions in the body behave analogously to potassium, which means that the cesium ions are located in the every cell of the body. Because of this specific trait radioactive cesium has no critical organ and is the so-called organotropic radionuclide.

Resorption of radioactive cesium, from the digestive tract of animals, is significantly reduced in animals that eat dry bulky feed/roughage.

Experiments showed that sheep which were fed dry bulky feed/roughage - alfalfa hay – were by 50% less radioactively contaminated with radioactive cesium from those that were fed pelleted feed. Also, the grazing nutrition causes a higher radioactive contamination with radioactive cesium of dairy cows compared with the indoor nutrition, this is highly expressed in ruminants, particularly in sheep, because they are taking parts that are close to the ground, resulting in entry of smaller amounts of land into body. Cows which are grazing in highland area are much more contaminated with radioactive cesium, compared with cattle that grazed in the plains: in the lowland/hilly-mountainous area by 7 times, and highland area by 30 times. Here, we should keep in mind the fact that is of crucial importance for the level of activity of radionuclide ^{137}Cs: the amount of precipitation in the highland area is 1.1 to 1.5 times higher than in lowland areas.

Distribution of radioactive cesium after a one-time entry into the body of cattle, sheep and pigs is similar in all species and approximate to distribution of potassium. However, the biological elimination is very different in the individual animal species and is significantly greater in

ruminants than in non ruminants. Biological elimination of the radionuclide ^{137}Cs is done with excretions (feces, urine) and secretions (milk) and during the first 7 days it is 30 % in feces and urine and 10 % in milk. Although the elimination of the radionuclide ^{137}Cs through milk is less studied than the elimination of radioactive strontium, it was known that 10 times more cesium is excreted through the milk than strontium, which appears to be higher, depending on the absorption of cesium from the digestive tract.

In case of multiple (daily) ingestion of radionuclide ^{137}Cs, over longer period of time, daily extracted milk quantities of dairy cows contain about 10 % of the daily entered activities of this radionuclide, which is an average 1.4 % per liter of milk. In dairy cows, the daily ingestion of fresh forages (clover) which had been radioactively contaminated with radioactive cesium - ^{134}Cs and ^{137}Cs - at the time of acute radiation situations related to the Chernobyl in 1986, the examination has shown that the level of activity of this radionuclide in the milk increases during the first 5-7 days, after which it declines slightly and remains at the saturation level. Almost identical results were obtained during the winter feeding of dairy cows with silage contaminated with radioactive nuclides of cesium - ^{134}Cs and ^{137}Cs *(IAEA, 1986a,1986b, 1996).*

Radioactive iodine

Radioactive iodine is a fission product with significant fission yield of about 4%. Radioactive iodine is introduced in the body of mammals in elemental form or as iodide, a smaller part directly from the air - as radioactive aerosols - but mostly indirectly through green food that is contaminated with radioactive atmospheric precipitation (Table 3).

Table 3. Characteristics: ^{131}I

Radionuclides	Type of radiation	Maximum energy (MeV)	The half-life		
	ß	0,2 0,6	Physical ($T_{½}$)	Biological ($B_{½}$)	Effective ($E_{½}$)
^{131}I	γ	0,08 0,28 0,36 0,63	8 d	40 – 180 d	7,5 d

Total of 90% of the radioactive iodine in food is absorbed and enters the bloodstream, where 30-50% is taken by the thyroid gland (^{131}I is called "Tireotropic radionuclide") and fixed to its saturation, and the remaining amounts are excreted from the body through milk and eliminated with urine and feces.

While radioactive contamination of animal feed with radioactive iodine has no large biological and practical importance, because ^{131}I has a short half-life, however, it is essential to know the fate of radionuclides in the body of dairy cows after single and repeated ingestion (*Martin et al. 2003*). Biological stream of absorption and distribution is shown in Figure 6.

After a single ingestion of ^{131}I, the radionuclide accumulates in the thyroid gland and 3 weeks after radioactive contamination there is still 87% of the total activity present in the body, while the other quantities of biological activity are eliminated in the urine, feces, and extracted through milk. Significant is the fact that after oral administration of the radioactive iodine into the organism of dairy cows, 30 minutes after ingestion it is possible to determine the radionuclide, and after 3-4 hours it reaches its maximum concentration.

Biological elimination of ^{131}I within 7 days of ingestion is: 50% in the urine, faeces 20% and milk 8%. Long-term daily ingestion of radioactive iodine increases rapidly its concentration in the thyroid gland, milk, urine and faeces, in order to reach a constant level after 5-10 days. The total amount of radioactive iodine, which is excreted in milked milk daily shows some variation and depend in particular on milk yield, milk - direct proportion - and also the time of year. The average can be calculated that the daily amount of excreted radioactive iodine is 1% per liter of milk. Radioactive iodine is contained here in more than 90% as iodide and may be completely removed from the milk using the ion exchange.

So we can conclude that milk and its products are radioactive vulnerable immediately after a nuclear accident *(Mitrovic,1995a, 1995b; Mitrović et al., 1995c)*. This is the "acute radiation situation", so called "iodine" phase, followed by occurrence of "chronic radiation situation."

Transfer of biologically important radionuclides from the plant foods in the milk

Transfer of biologically important radionuclides from plant food, i.e. grass species from pastures to milk, compared to other foods of animal origin, is of particular interest, since radionuclides from plant foods that contain them soon pass into milk that is continuously produced *(Fulker et al., 1989, Crount et al., 2003)*. Milk is a food that is very important especially for vulnerable populations such as infants and young children and the elderly and the sick, and therefore great attention is paid to the origin and quantity of all types of residues in milk. The

radiation load of milk with radioactive residues is almost exclusively a consequence of the transfer of radionuclides in the food chain, i.e. in the segment soil - plants - milk (*Alberts et al., 2000).*

In recent years, based on knowledge of the distribution of biologically important radionuclides in certain milk components, using the usual methods of fermentative processing of milk it was managed to get out of the casein complex radionuclides ^{90}Sr, ^{137}Cs while ^{131}I remains tied almost exclusively in the fat component of milk. So using adequate method, large amounts of useful dairy products can be obtained, while butter has to sit some time in order for radionuclide ^{131}I to decay below the allowed limit of radioactive activity.

Material and methods

Total sampled were 8 samples of milk and cheese from individual manufacturer. Milk samples were collected as a bulk sample of milk obtained from dairy cows, the same applies for cheese samples. In addition, milk samples were collected in special plastic bottles of 1 liter volume, and samples of cheese in plastic bags in quantities of 1 kg. The above samples transferred to the hand fridge in the lab for testing.

Gamma spectrometry analysis and measuring the ^{137}Cs activity concentration in the food chain was performed on the detector of high purity germanium (HP Ge) with a multichannel analyzer ("ORTEC``). Examinations were performed in laboratory conditions.

The aim was to investigate whether one of the factors that can potentially affect the radiation-hygienic variations between natural and artificial radionuclides, and therefore their process of selection and elimination, was the mutagenic potential of radioactivity present in these foci.

The results were statistically analyzed and discussed in comparison with the findings of other authors.

Results and Discussion

The results represent the values for the total sample of milk or cheese for each manufacturer. Table 1 shows the measured values shown according to the type of sample and the sign of the sample.

Activity of milk was from 0.09 to 0.66 Bq / kg, or an average of 0.40 Bq / kg. It is notable that the relevant activities of milk transferred to the cheese in all eight samples. Thus, the results of activities in the cheese ranged from 0.07 to 0.47 Bq / kg, or an average of 0.26 Bq / kg (Table 4).

Table 4. RH analysis of samples of milk and cheese of Golija for ^{137} Cs (Bq/kg)

Sample	Values of the specific activity ^{137} Cs (Bq/kg)								
	Number of samples								Xsr
	1	2	3	4	5	6	7	8	
Milk	0,43	0,23	0,65	0,09	0,32	0,35	0,66	0,54	0,40
Cheese	0,21	0,19	0,43	0,07	0,30	0,22	0,47	0,26	0,26

Activity of milk and cheese was in the range from 0.07 to 0.66 Bq / kg, which is confirmed by the results of monitoring radioactivity in the environment *(Pantelic et al., 2007)*. Similar results were obtained in the study by *Javorina et al. (2001, 2007)* where ^{137}Cs activity in the range of measured background. In the study by *Jankovic et al. (1999)* measured milk samples also have values greater than 1BQ. Results of veterinary-sanitary control *(Vukovic et al., 1997)* confirmed that milk as a strategic foodstuff is safe/correct from the radiation-hygienic aspect also when imported.

Contamination of the biosphere with radionuclides in most part was contributed by the atmospheric precipitation of a nuclear explosion. A significant part of the fission products came to the stratosphere where it is transported to long distances. Deposition of radionuclides from the stratosphere is a significant source of global contamination of soil. The most important radionuclide from this source is ^{137}Cs.

Radioactive isotope ^{137}Cs was introduced into environment by precipitation, after the nuclear tests in the 60`s and after the nuclear disaster at Chernobyl 1986. Chemical similarity to potassium explains its high mobility in biological systems. Because of its long half-life ^{137}Cs ($T_{1/2}$ of 30 years) it must be monitored in all samples of the environment *(Pantelić et al., 2007*). Thus, transfer of Chernobyl cesium into the milk of cows was compared in the various Nordic countries. Data were collected from agricultural and individual farms. In years 1986 and 1987 the highest amounts of ^{137}Cs were found in Finland and Norway, the average value in Sweden, the Faroe Islands and Iceland, and the lowest values were detected in Denmark.

Rinsing process and relocation of cesium can lead to very uneven distribution of radionuclides in one area so that based on the results of numerous

studies it can be concluded that the migration of radionuclides in the soil depends on a number of environmental factors, especially soil properties such as physical-chemical properties (content of organic matter, adsorption properties of the complex, pH, mineral composition, etc..), structure (particle size distribution, attention), water regime (water content, groundwater level), cultural practices (tillage, fertilization), etc., *(Sekulic et al., 2003)*. Also a model was made predicting the concentration of ^{137}Cs in cow's milk based on soil characteristics and quantity of radioactive fallout in England *(Gillet et al., 2001)*.

Conclusion

- According to the the measured values and the overall results of the measurements of radioactivity on mountain Golija it can be concluded that the activities of natural and artificial radionuclides in milk and dairy products ranged within limits that are characteristic for these areas.
- In these key samples in the food chain of dairy cows, establishment of critical control points at selected locations, as concerns the level of activity of produced radionuclides, would ensure quality food in terms of radiation-hygienic supervision. Such nutrition provides the production and radiation-hygienic correct milk as food and raw material for further processing.
- Radiation-hygienic supervision over the HACCP system in the region of Golija guarantees radiation safety processes of primary production of milk and dairy products - cheese.
- Quantitative determination of the resources status of Golija mountain from the radio ecological aspect in the production of healthy food.
  System optimization in radiation-hygienic supervision and monitoring at key points of the food chain of dairy cows, production and processing of milk.
- Contribution to the further optimization of the monitoring of radioactivity produced, which is of particular importance for other types of agricultural production (plant and animal).

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Bezbednost golijskog sira i mleka sa radijaciono-higijenskog aspekta

M. Vićentijević, R. Mitrović, V. Vuković

Rezime

U procesu proizvodnje, obrade i prerade mleko se pored biološke kontaminacije (mikroorganizama) može zagaditi i mnogim abiotičkim materijama. Te materije su proizvod čovekove delatnosti. Potiču iz spoljašnje sredine i u mleko dospevaju kao posledica nesavršenosti tehnologije proizvodnje mleka, a u mnogim slučajevima nemoći i neznanja aktera mlekarske industrije da spreče kontaminaciju. U higijeni mleka naročitu pažnju treba obratiti na radioaktivne materije. U radu su izneti rezultati rada Laboratorije za radijacionu higijenu - »LABRAH» pri Naučnom institutu za veterinarstvo Srbije u Beogradu. Radijaciono-higijenska kontrola mleka i sira sa područja Golije izvršena je gamaspektrometrijskom analizom. Rezultati koje smo dobili jasno ukazuju da je proizvodnja mleka i sira bezbedna i u granicama fona za odgovarajuće podneblje.

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HERITABILITY AND REPEATABILITY ESTIMATION OF MILK YIELD TRAITS OF BLACK AND WHITE COWS

D. Stanojević[1*], R. Đedović[1], V. Bogdanović[1], R. Beskorovajni[2], P. Perišić[1], M. Popovac[1], N. Popović[3]

[1]University of Belgrade, Faculty of Agriculture, Institute for Zootechnics
[2]PKB INI Agro-economic Padinska Skela-Beograd
[3] Institute of science application in agriculture, Belgrade
Corresponding author: [*]Dragan Stanojević, stanojevic@agrif.bg.ac.rs
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Abstract: The aim of this study was genetic parameter estimation of milk yield traits of black and white cows. This research was conducted on 5 835 cows that had finished their first three lactations in period from 1993 to 2010. Cattle were bred at 7 farms of the Agricultural Corporation Belgrade and they are descendants of 62 bulls. The following traits were researched: milk yield, milk fat content, milk fat yield and 4% fat corrected milk. Variance components were estimated using Restricted Maximum Likelihood (REML) of Statistical Analysis System (SAS, Version 9.1.3., 2012.). The estimation of the heritability for milk yield, fat yield, fat percentage and 4% fat corrected milk were 0.20, 0.11, 0.10 and 0.19 respectively for single models. Repeatability estimation for mentioned traits was 0.38, 0.27, 0.38 and 0.36.

Key words: milk traits, heritability, repeatability, black and white cows

Introduction

The efficiency of animal selection for milk production is directly proportional to an estimated breeding performance of the animals selected to be the parents of subsequent generation. In order to have the selection effect as greater as possible it is necessary to know the values of genetic parameters (heritability and repeatability) for the traits under a great selection pressure. In order to design a breeding programme that will yield satisfactory results, it is necessary to know the relationship between the additive and non-additive gene effects (*Kahia et al. 1999*).

Heritability coefficient is a relationship that may change with every change of numerator and denominator (*Đedović et al. 2012; Stanojević et al. 2012*). However, changes in gene frequency caused by selection on milk yield traits are

minor ones for most of the genes, therefore the simultaneous changes in heritability can be difficult to register. In smaller populations the inbreeding seems to be inevitable and it is even intensified by selection. Because of that the homozigosity may increase while the heritability may decrease (*Stanojević, 2012*). It is obvious that the inbred animals are less equalized against environment fluctuations, therefore the environment variance may be increased while simultaneously decreasing the heritability. Any increase in the environmental variance not in harmony with proportional increase in genetic variance may also reduce the heritability.

Heritability estimation based on sire components may result in lower heritability than that obtained by way of variance components "among the sires in the herd". Similarly, not taking into account the age effect, it is perceived that drop in heritability values is reduced. This might be caused by differences between the heritability estimations for milk yield. Some studies are based on the results obtained by cows of the same age, however most studies are based on the age corrected results obtained by cows of various age.

Some reports (*Đedović, 2000*) also show different heritabilities for different level of nutrition, care and health protection. It is assumed that genetic differences are reduced due to bad conditions since their deficiency shows tendency to keep the animals at a comparable low level of production increasing at the same time the environment variance while decreasing the heritability.

A great number of traits of primary importance regarding the milk production, such as lactation duration, milk yield, milk fat content and protein content can be measured many times during an animal productive life. On the basis of greater number of measurements made for one trait in the same animal, it is possible to calculate the repeatability coefficient for given trait and thus to increase the breeding value estimation accuracy. In addition, the repeatability coefficient is used for calculating an animal potential production ability (MPPA) on the basis of the first production results. Ranking of cows on the basis of their expected production ability is an efficient way to collect the information on secretion.

The objective of this research was to establish the heritability and repeatability coefficients values for the milk traits having a primary importance for milk production.

Material and Method

The heritability and repeatability coefficients values were calculated on a set of records involving production results of 5 835 black and white cows. The animals were bred on 7 farms of the Agricultural Corporation Belgrade in the

period from 1993 to 2010. The animals were the descendants of 62 bull-sire and all the animals had finished the first three standard lactations.

The values of genetic parameters were calculated for following milk yield traits in standard lactation: milk yield (MY-305), milk fat content (FC-305), milk fat yield (FY-305) and 4% fat corrected milk yield (4%FCM-305). The variance components were calculated by means of REML methods within the VARCOMP procedure (SAS 9.3, 2012). In order to estimate the effects of fixed and random factors the following mixed model was used:

$$Y_{ijklmn} = \mu + O_i + F_j + G_k + S_l + L_m + H_p + e_{ijklmp},$$

where:

Y_{ijklmn}- is a phenotype expressiveness of the examined trait,

O_i- random effect of bull sire (i= 1....62),

F_j- fixed effect of the farm on which the animal realized production (j=1...7),

G_k- fixed effect of calving year (k=1...17),

S_l- fixed effect of calving season (l=1...4),

L_m- fixed effect of lactation in order (m=1...3),

H_p- fixed effect of the share of genes of Holstein-Friesian breed (p=0....100),

e_{ijklmp}- random error.

The coefficients of heritability (h^2) and repeatability (r) included in the study were calculated by means of the following pattern:

$$h^2=\frac{\sigma_a^2}{\sigma_p^2} \qquad \text{and} \qquad r=\frac{\sigma_a^2+\sigma_{pe}^2}{\sigma_p^2}$$

where:

σ_a^2- is the additive genetic variance,

σ_{pe}^2- variance of permanent effect of environment factors,

σ_p^2- total (phenotypic) variance, composed of additive genetic variance, dominance variance, epistasis variance, variance of permanent effects of environment factors and error variance ($\sigma_p^2 = \sigma_a^2 + \sigma_d^2 + \sigma_i^2 + \sigma_{ep}^2 + \sigma_{pe}^2 + \sigma_e^2$).

Results and Discussion

Table 1 shows the indicators of phenotype expressiveness and variability of milk yield traits involved in the analysis:

Table 1. Indicators of phenotype expressiveness and variability of milk yield traits in standard lactation

Traits	n	$\bar{x}$	SD	Cv	min	max
MY-305	24 447	7 241	1 716	23.7	2 169	19 180
FC-305		3,56	0.30	8.4	2,04	5.74
FY-305		257	59.1	23	101,32	788.93
4%FCM-305		6 744	1544	22.9	2107	19028

Animals bred in the analyzed period produced on average 7 241 kg milk containing 257 kg milk fat. An average yield of 4% fat corrected milk was 6 744 kg. The established mean values for milk yield are significantly higher than the values established in the studies by *Đedović (2000, 2012)*, *Stanojević at al.2012*, *Trifunović et al. (1998*) *and Mitsouyoshi et al. (1994),* but they agree with the results obtained *by Carlen et al. (2004)* and *Cienfuegos-Rivas et al. (1999).*

Table 2 displays the values of the variance components for examined factors as well as the values of heritability and repeatability coefficients:

Table 2. Values of heritability (h^2) and repeatability (r) coefficients

Trait	σ_a^2	σ_{pe}^2	σ_p^2	h^2	r
MY-305	465192.206	406177.351	2283337.383	0.2037	0.3816
FC-305	0.007	0.018	0.065	0.1077	0.3846
FY-305	315.046	467.574	2858.725	0.1102	0.2738
4%FCM-305	369250.65	331755.51	1947239.336	0.1896	0.3600

The heritability coefficient values for examined milk yield traits were from 0.1077 for milk fat content to 0.2037 for milk yield in standard lactation. Confirmed heritability values were lower than those obtained by *Mitsouyoshi et al. (1994)* and *Hammami et al. (2008)* when they investigated the population of Holstein-Friesian cows in Luxembourg, but they comply with the results for Holstein-Friesian breed in Tunisia, confirmed in the same study of these authors, and also with the results obtained in the study by *Gorbani et al. (2011)*. Obtained heritability values for milk yield are higher than those obtained in the

investigations of *Yang et al. 2005, Strabel and Jamrozik 2006,* and *Espinoze et al. 2007*.

Relatively low values of heritability coefficients confirmed for milk yield traits in standard lactation can be explained by the use of limited number of bulls from relatively small number of lines, what resulted in reduced genetic variability of production traits.

The values of repeatability coefficients were from 0.2738 for milk fat yield to 0.3846 for milk fat content. Confirmed repeatability coefficients values are in harmony with the values obtained in investigations by *Gorbani et al. (2011)*, but they are lower than values obtained by *Mitsouyoshi et al. (1994)*.

The significantly higher values of repeatability coefficients in relation to heritability coefficients established in this study can be explained by a significant effect of environment permanent factors. Namely, the animals included in the analysis were kept on 7 typical farms situated in almost identical micro geographical region. Moreover the way of keeping the animals was the same on all examined farms, with almost the same composition of ration and the same feed quality, therefore it is to be expected that studied factors had a permanent and, at the same time, significant effect on the variability of studied traits.

Conclusion

The mean values for milk yield traits in standard lactation does not deviate significantly from the results obtained for other populations of black and white breed by other authors, indicating a high genetic potential possessed by our population.

Confirmed lower values of additive genetic variance suggest a dominant effect of environment factors on the expressiveness and varying of milk yield traits in standard lactation. In addition, higher values of repeatability coefficients in comparison with the heritability coefficients are the consequence of a considerable effect of permanent environment factors on the studied traits. In the next period it is necessary to pay the attention to the increase in genetic variability by using a greater number of bulls from different lines in order to increase the values of heritability coefficients and therefore the selection effect as well. It is also necessary to work on the improving of environment factors so that the realized genetic advancement could be applied as well.

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Ocena vrednosti heritabiliteta i repitabiliteta osobina mlečnosti crno-belih krava

D. Stanojević, R. Đedović, V. Bogdanović, R. Beskorovajni, P. Perišić, M. Popovac, N. Popović

Rezime

Cilj ovog istraživanja bio je da se procene vrednosti genetskih parametara za osobine mlečnosti crno-belih krava. Istraživanjem je obuhvaćeno 5 835 krava koje suimale zaključene prve tri laktacije u period 1993 do 2010. Godine. Grla uključena u istraživanje gajena su na 7 farmi Poljoprivredne korporacije Beograd i bile su potomci 62 bika oca. Istraživanjem su obuhvaćene sledeće osobine mlečnosti u standardnoj laktaciji: prinos mleka, sadržaj mlečne masti, prinos mlečne mast i prinos 4% mast korigovanog mleka. Komponente varijanse izračunate su korišćenjem metoda REML u okviru statističkog softvera SAS (Version 9.1.3., 2012.). Utvrđene vrednosti koeficijenta heritabiliteta za prinos mleka, sadržaj mlečne masti, prinos mlečne mast i prinos 4% mast korigovanog mleka iznosile su 0.20, 0.11, 0.10 i 0.19. Koeficijenti ponovljivosti za posmatrane osobine imali su vrednosti: 0.38, 0.27, 0.38 i 0.36.

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QUALITY CONTROL OF MILK AND MILK PRODUCTS BASED ON PHYSICOCHEMICAL PROPERTIES

B. Pisinov[1], A. Kasalica[1], V. Vuković[2]

[1] JPS Dairy Institute, Auto put 3, 11070 Novi Beograd, Republic of Serbia
[2] Scientific Veterinary Institute of Serbia, Auto put 3, 11070 Novi Beograd, Republic of Serbia
Corresponding author: boriss752002@yahoo.com
Communication

Abstract: Milk and milk products, as important foodstuff of animal origin, must meet the requirements in term of quality. Among those requirements are the physical and chemical properties, including the appropriate parameters, depending on the type of product, declared composition, etc. Accredited laboratory of JPS Dairy Institute, in 2012 examined milk fat and protein content in 140 samples of pasteurized and UHT milk, and from fermented milk products in testing were included: yogurt, sour milk, fermented milk products with probiotic bacteria and fermented dairy drinks. In addition, in 65 samples was determined: milk fat, dry matter and pH. Also, there were analyzed 280 samples of cheese: ripening cheeses and cheeses without ripening, where the following quality parameters were examined: milk fat, dry matter, milk fat in dry matter and water in fat-free matter. Based on the results, quality assessment of products was performed.

Key words: milk, milk products, physicochemical examination, parameters, quality control

Introduction

As foods of animal origin, milk and milk products are among the more important, because of its nutritional value, but also because they are particularly important in the diet of children and older people, as separate categories. It should be mentioned how important it is that these foods are correct and in agreement with a number of regulations in terms of quality. Firstly, it refers to the presence of hazardous substances, microbiological and hygienic correction, sensory properties, and significant requirements include chemical and physic-chemical properties. Also very important is the frequency and continuity to carry out investigations, using appropriate equipment, methods and testing procedures. Of course, depending on the type of sample and tests, regulations and testing procedures are matched for the same.

Narrowing the issue to chemical and physic-chemical properties of milk and milk products, assay included various types of samples, where were examined appropriate parameter.

In mentioned samples, the quality should be satisfied for individual categories and types of product, which has been the subject of research by many authors such as: *Miočinović (1993), Miočinović et al. (1996), Milanović (1997), Carić (1997), Topisirović and Ostojić (2005), Miočinović et al. (2008)* and others.

The investigation included samples intended for the market, namely: heat treated milk, fermented milk products with and without probiotic bacteria and fermented dairy drinks. Also cheeses with and without ripening are included. In each of the above mentioned products are tested appropriate parameters in order to determine the quality, constancy of control and performing categorization. It should be noted that the tests were conducted at the request of the manufacturer and the inspection in continuity of the one-year period and to provide a credible quality picture of tested products.

Materials and Methods

Accredited laboratory of JPS Dairy Institute during 12 months analyzed samples of milk and milk products intended for the market, distributed from 10 industrial dairies for quality control testing. All samples were produced from cow's milk.

Whereby, the heat-treated milk testing included 140 samples, which were 75 samples of pasteurized milk and 65 samples of sterilized milk. In these samples were examined content of milk fat and protein.

The examination of fermented milk products was included: yogurt, sour milk, fermented milk products with probiotic bacteria (probiotic yogurt) and fermented dairy drinks (fruit yogurt). A total of 65 samples were tested in which were determined: milk fat, dry matter and pH values. Analyzed samples were: 34 yogurts, 10 sour milks, 16 probiotic yogurts and 5 fruits yogurts.

A total of 280 cheese samples were examined as 199 ripening cheeses and 81 cheeses without ripening. In these samples were tested content of milk fat, water, milk fat in dry matter and water in fat-free matter.

Milk fat content in heat-treated milk and fermented milk products is tested according to *ISO 2446:2008* and for cheese method *ISO 3433:2008* was used. For the determination of protein in heat-treated milk, a method *ISO 8968-2:2001* was applied, using digestion system (Buchi – K 424 TG, Switzerland) and distillation system (Vapodest 20s, Germany). Dry matter content of fermented milk was determined by *ISO 13580:2005*, and method *ISO 5534:2004* was used for cheeses. pH value as physic-chemical parameter was determined by *AS 2300.1.6:2010* with

electrochemical analyzer (Consort C860, Belgium). The values of milk fat in dry matter and water in fat-free matter were obtained by calculation procedure.

Based on the results, the quality of the products was evaluated, as well as categorization of cheeses under applicable Regulations (*Službeni glasnik RS, 33/10*), statistical analysis of the results was applied.

Results and Discussion

During the annual research, analyzed samples were heat-treated milk, fermented milk products and cheeses. Test results are given in stated tables.

Chemical analysis of the pasteurized milk with 2.8% fat and 3.2% fat, in question of the milk fat, shows that the quality of all tested samples is properly, as the protein content of milk with 3.2% fat, while in milk with 2.8% fat was not the case (97.8% appropriate). On the other hand, for sterilized milk samples, proper was 96.9% and 98.5% of the samples in terms of content of milk fat and protein (Table 1).

Table 1. Results of chemical analysis of heat treated milk

Samples	Number of samples	Milk fat (%)		Protein (%)	
		Correct samples		Correct samples	
		Number	%	Number	%
Pasteurized	75	---	---	---	---
- with 2,8% mf	45	45	100.0	44	97.8
- with 3,2% mf	30	30	100.0	30	100
Sterilized (2.8% mf)	65	63	96.9	64	98.5
Total	140	138	98.6	138	98.6

mf – milk fat

The results of chemical testing of pasteurized milk quality in the period from 1990-1992., showed a significantly higher percentage of unsatisfactory samples (65.07-71.22%) in terms of non fat solids *(Miočinović, 1993)*.

Results of chemical and physic-chemical testing of yogurt and fermented dairy drinks responded in terms of all measured parameters. It is same for dry matter content of sour milk and fermented milk products with probiotic bacteria. Percentage of appropriate samples, in terms of milk fat was 80.0% for sour milk and 93.8% for fermented milk products with probiotic bacteria. Relating to pH value, 90.0% samples of sour milk were appropriate, while the value of fermented milk products with probiotic bacteria was 93.8% (Table 2).

Table 2. Results of physico-chemical analysis of fermented milk products

Parameter	Findings		Yogurt	Sour milk	Fermented milk products with probiotic bacteria	Fermented dairy drinks
	Number of samples		34	10	16	5
Milk fat (%)	Correct samples	Number	34	8	15	5
		%	100.0	80.0	93.8	100.0
Dry matter (%)	Correct samples	Number	34	10	16	5
		%	100.0	100.0	100.0	100.0
pH value	Correct samples	Number	34	9	15	5
		%	100.0	90.0	93.8	100.0

According to *Miočinović (1993)* in fermented milk products, the percentage of unsatisfactory samples ranged from 33.08-51.38%, while for the period 2005-2008., *Miočinović et al. (2008)* found 8.13% unsatisfactory samples. In recent years, probably technological advances and increased control leads to significant reduction in defects of fermented milk products. That was shown in our results of 7.7% unsatisfactory samples of fermented dairy products.

The analysis of ripening cheeses in terms of milk fat, showed 100.0% appropriated samples, which is also valid to dry matter in cheese without ripening. In contrast, only 59.3% of results for cheeses without ripening are suited to the content of milk fat. This percentage, in ripening cheeses in terms of dry matter was 99.5%. In ripening cheeses, based on the fat in dry matter (FDM) and water in fat-free matter (WFFM), it can be conclude 100% of correctness as required by the Regulations. In cheeses without ripening, significant differences were obtained in the content of FDM, where it was appropriate only 59.3% of the samples. A total of 99.5% of ripening cheese samples, were in accordance with the Regulations in terms of WFFM (Table 3).

Table 3. Results of chemical analysis of cheese

Parameter	Findings		Ripening cheese	Cheese without ripening
	Number of samples		199	81
Milk fat (%)	Correct samples	Number	199	48
		%	100.0	59.3
Dry matter (%)	Correct samples	Number	198	81
		%	99.5	100.0
Fat in dry matter (%)	Correct samples	Number	199	48
		%	100.0	59.3
Water in fat-free matter (%)	Correct samples	Number	198	81
		%	99.5	100.0

According to research by other authors, the chemical composition of the cheeses showed a large quality variation in recent decades. In tests conducted during 1990-1992, the quality of industrially produced cheeses shows complete correctness in required parameters, while white cheeses from individual producers were incorrect in 88.23% *(Miočinović, 1993)*. In the period from 1993 - 1996, the quality of cheeses were regular in 82.22% of samples, and incorrect in 17.78%, of which the hard cheeses had the greatest variation in terms of increased water content in fat-free matter (56.06%), and water (46.67%) *(Miočinović et al., 1996)*. Our findings, when compared with the results of the above research, show a noticeable improvement in the cheese quality of water in fat-free matter and total solids content, which was confirmed in 99.5% of samples. *Ostojić (2010)* examined the chemical composition of ripening cheeses from Golija region. The obtained results indicate a very good quality production where demanded quality accomplished 100% of samples in total solids content, FDM and WFFM.

Based on the results of cheeses without ripening, it can be concluded significant deviation from the qualities requirement, where 40.7% of samples did not correspond to the contents of fat and fat in dry matter.

Conclusion

Based on chemical and physic-chemical results for milk and milk products, it was obtained a total of 12.6% of unsatisfactory samples, of which 10.7% were cheeses without ripening. It is notable that in recent years there was a significant improvement of the quality of milk and milk products, excluding incorrect samples of cheeses without ripening. Thus, from 433 examined samples, 98.85% was correct. Probably, the industrial origin of samples contributed to high degree of

correct samples because of rigorous control of the production technology (standardization process).

Kontrola kvaliteta mleka i proizvoda od mleka sa fizičko-hemijskog aspekta

B. Pisinov, A. Kasalica, V. Vuković

Rezime

Mleko i proizvodi od mleka, kao značajne namirnice animalnog porekla, moraju da zadovoljavaju propisane uslove u pogledu kvaliteta. Među te zahteve spadaju i fizičko-hemijska svojstva, uključujući odgovarajuće parametre, u zavisnosti od vrste proizvoda, deklarisanog sastava itd. Akreditovana laboratorija JPS Instituta za mlekarstvo, u 2012. godini ispitala je sadržaj mlečne masti i proteina u 140 uzoraka pasterizovanog i sterilizovanog mleka, a od fermentisanih proizvoda od mleka ispitani su: jogurt, kiselo mleko, fermentisani proizvodi od mleka sa probiotskim bakterijama i fermentisani mlečni napici. Pri tome, u 65 uzoraka određeni su: mlečna mast, suva materija i pH vrednost. Takođe, analizirano je i 280 uzoraka sira: sireva sa zrenjem i sireva bez zrenja, kod kojih su ispitani parametri kvaliteta: mlečna mast, suva materija, mlečna mast u suvoj materiji i voda u bezmasnoj materiji. Na osnovu dobijenih rezultata, izvršena je procena kvaliteta proizvoda.

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BIOFUEL INDUSTRY BYPRODUCTS – ALTERNATIVE OF TRADITIONAL PLANT PROTEIN SOURCES IN RUMINANT' DIETS

L. K. Kozelov, M. R. Yossifov

[1] Institute of Animal Science Kostinbrod, Bulgaria
Corresponding author: m_vet@abv.bg
Original scientific paper

Abstract: The biodiesel and bioethanol' production constantly increasing worldwide. In this regard, increased its byproducts. The aim was to evaluate and compare the main byproducts from biofuel-industry (rapeseed meal (RSM) and dried distillers' grains with solubles (DDGS)) as a protein source with vegetable origin. The paper is based on both, literature overview and our own data. The target was ruminant species from different categories. It's conducted a series of trails (laboratory, balance and feeding) with following indexes: chemical composition, feeding value, animal performance, etc. As a whole, results were affected positively and tested forages could be used as an alternative to conventional (sunflower meal, soybean meal, etc.) protein concenttrates. Based on these data we made the following more important conclusions:

Rapeseed meal (RSM) and DDGS, produced in Bulgarian, aquality were no significant differences from other ones worldwide;

Rapeseed meal containing minimal concentration anti-nutritive factors (glucosinolates – up to 30 μmol.g^{-1}) and erucic acid – up to 2 %) and could be recommended as protein and / or energy source in the rations of ruminants without restriction;

Recommended 10 % RSM inclusion in diets of lactating dairy ewes is optimal, and for fattening lambs could be overstated;

DDGS inclusion in ruminant' diets could be up to 40 percent.

Keywords: biofuel, feed, sheep, DDGS, rapeseed meal (RSM), ruminant, Bulgaria

Introduction

Actuality of the problem. An intensified search for alternatives to traditional petroleum fuels is on hand worldwide. Leaders in this category now are so called biofuels yielded by „energy crops" as cereals and oilseeds. In this regard,

there is an enormous expansion of areas planted with these crops. Main raw material in Bulgarian biodiesel industry is rapeseed and in bioethanol – corn and wheat. According to European Union (EO) Directives and adopted Bulgarian national strategy in 2008 the usage of biofuels in the EU countries should gain minimum up to 2 % of the total fuel consumption, in 2010 – 6 %, in 2020 – 10 % and in 2030 – 25 %. According to long-term plans biofuels comsumption in 2050 is estimated *(Matthes et al., 2011)* up to 80%.

Rapeseed and processing by-products. The term "*biodiesel industry by-product*" covered all kind of residues (by-products, subproducts and co-products) resulted in parallel with the main product (biooil). They're secondary or concomitant product in technological scheme and often are considered as a tool to gain additional profits for manufacturers or reduce the cost of the main product *(Fabiosa et al., 2009)*.

Worldwide. EU is a leader in the oilseed rape production worldwide (table 1). It's producing about one third of the total yield (33 – 35 %). Second place was shared by Canada and China, followed by India. During the previous 2012 has been registered the greatest increment in USA' yields (14 %) followed by India (10 %) and EU (4 %). Negative trends in Ukraine, Russia and Bulgaria are due to adverse weather conditions during the previous year (winterkill and dryness). On an annual basis, the recorded global yields are up to 4 %. Simultaneously, it's expected global rapeseed production 2013/14 to reach a weight of 65.3 MT (*www.informaecon.com*). An expected 7 % augmentation included raises in EU - 27 with 20.8 MT, Canada - 16.5 MT, China - 12.8 MT, India - 6.9 MT. These growth rates are due to 12 % increasement in Canada and near 5 % in EU_{27} and India's rape production.

Table 1. Trends in rape production (million tons) *

Country	2009	2010	2010/09, %	2011	2011/10, %	2012	2012/11, %
EU - 27	21.7	20.6	-5.1	19.1	-7.3	19.9	+4.2
Bulgaria	**0.2**	**0.6**	**+129.2**	**0.6**	**+1.8**	**0.27**	**-51.8**
Russia	0.7	0.7	0.0	1.0	+42.9	1.0	0.0
Ukraine	1.9	1.5	-21.1	1.4	-6.7	1.1	-21.4
Canada	12.9	13.1	+1.6	14.2	+8.4	14.7	+3.5
USA	0.7	1.1	+57.1	0.7	-36.4	0.8	+14.3
China	13.3	12.2	-8.3	11.6	-4.9	12.0	+3.4
India	6.0	7.1	+18.3	6.3	-11.3	6.9	+9.5
Australia	1.9	2.4	+26.3	3.0	+25.0	2.8	-6.7
Other	2.1	1.8	-14.3	1.9	+5.6	1.9	0.0
Total	61.3	60.7	-1.0	59.5	-2.0	61.5	+3.4

* www.aocs.org

EU 27. The main producers in the countries of EU_{27} are Germany, France and Poland. Results in recent years are hesitant in this area due to adverse weather conditions (drought, frost). Planted and harvested areas (*www.thebioenergysite.com*) varied about 7.0 M ha and production is counted about 20 MT (table 2). Observed decline in the amount of planted areas (4 %) at the expense of increased yield (2 %). At the same time, imported quantities rape decreased (7 %) at the expense of an increased percentage of crushed rape in the countries of EU_{27} (2 %). The rape is the main crushing oilseed in EU_{27} (near 22 MT), followed by soybean (near 11 MT) and sunflower (near 7 MT). Ukraine (from Europe), Canada, Australia (from world) are the main partners for EU import (*www.thebioenergysite.com*). The presented quantities of rapeseed meal (table 2) correlate with the equation bellow [1]. The data of produced rapeseed meal 2012/13 showed a clear increase (2 %) after 3 percent decline in 2011/12. The popularity of this by-product as a part of the livestock' diet varies among different EU countries. The main factors are traditions in the rape crush and rate of development of high-productive dairy farming (Germany, France and United Kingdom). Imports increased in 2010 (67 %), but in recent years declined in this item (5 %).

Table 2. Trends in production of rape and rapeseed meal (RSM) in the countries of EU_{27}*

	2009	2010	2010/09, %	2011	2011/10, %	2012	2012/11, %
							Rapeseed
Area Planted	6.5	6.9	+6.2	7.0	+1.4	6.7	-4.3
Production	21.4	20.7	-3.3	19.1	-7.7	19.5	+2.1
Imports	2.1	2.6	+23.8	3.0	+15.4	2.8	-6.7
Crush	23.0	22.7	-1.3	21.7	-4.4	22.1	+1.8
							Rapeseed meal
Production	12.5	12.6	+0.8	12.1	-4.0	12.3	+1.7
Imports	134.0	224.0	+67.2	220.0	-1.8	210.0	-4.5
1000HA, 1000MT							* ww.thebioenergysite.com

Bulgaria. Bulgaria occupies a middle position among the ranks of producing rape in EU_{27} (table 3). The production of this crop since the country's admission to the EU marks a sustained annual growth rate (at times). This growth till 2010 was accompanied by EU subsidies, but in the last few years and the constant demand for rape from West European *countries (*Germany, France) as biodiesel feedstock. Till now, has been putted into operation six large factories for biodiesel production. In a condition of competitive market, over the years only one of them managed to escape. Nowadays it disposes of a modern factory with a capacity of 60.000 T/year. There are also a number of small oil-factories without significant market share. Sector problems stem from lack of raw material cause of produced quantities rape are exported (Germany, France).

Table 3. Trends in production of rape in Bulgaria*

	2009	2010	2010/09, %	2011	2011/10, %	2012	2012/11, %
							Rapeseed
Area Planted	1.1	2.2	+100.0	1.9	-13.6	1.6	-15.8
Production	0.2	0.5	+125.0	NA	-	NA	-
1000 Mdka, 1000MT						*www.mzh.government.bg	

Available data on the produced quantities of rapeseed by-products from biodiesel industry are lacking, but in keeping with World and European trends this market shows relevance and gaining ever greater outlooks. The technological process (Fig. 1) in biodiesel production can be summarized in the following scheme (figure 1):

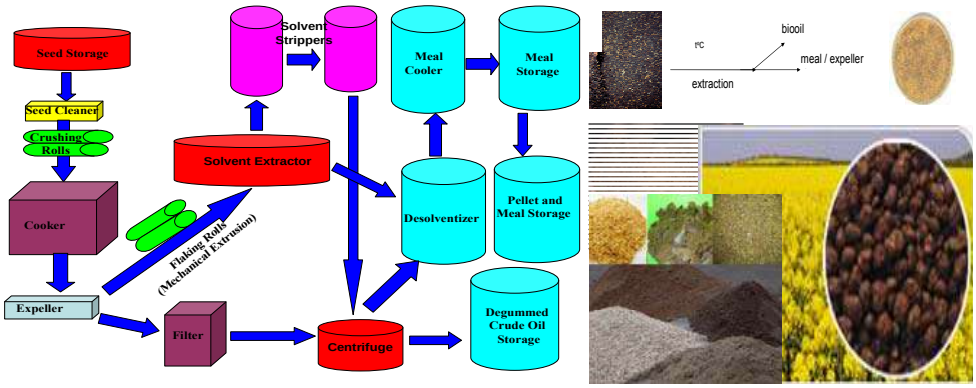

Figure 1. Technology of rapeseed meal (RSM)' production

The lack of statistic data in the country about the quantities of rape products (meal, expeller) can be compensated by using as a guide the following equation *(Schermerhorn et al., 1986)*:

1 T rapeseed –> 360.0 L bio-oil + 640.0 kg rapeseed meal, [1]

According to technology of oil-extraction *(Scheithauer et al., 1988)* in the country are available following rapeseed by-products:

- *Expeller* – by-product with up to 16 – 18.0 % residual oil as a result from mechanical extraction (pressing);
- *Meal* – by-product with residual oil < 3.0 % obtained by solvent extraction (hexane);

- *Extruded meal* - after a two-stage processing (pressing + chemical solvent) with < 1.0 % residual oil.

Insufficient scientific knowledge and farmers disinformation about the qualities of the rapeseed by-products as a source of protein and/or energy in high-productive farming. Also, the lack of adequate available amounts of this product led to low interest and undervalued. In the available scientific and popular literature lacks sufficient information both as regards of its characteristics (chemical composition and nutritional value) and its productive effects on different species and categories. Overviewed data about chemical, mineral and amino acid (AA) composition of RSM is presented in table 4. CP content varied in range 34 - 40 %. It's with high biological value and high level of rumen undegradable protein (RUP) or „bypass". Our own data *(Yossifov et al., 2011a)* showed good parity (36.4 %). AA concentration varied 1.6 - 4.7 and 1.5 -2.7 %, for Lys and Met + Cys respectively. Fat content was in close range (2.0 - 3.8 %) and our data was in lower range (3.1 %). Crude fiber content (9.9 - 13.7 %) varied among the origin and processing (dehulling, etc.).

Table 4. Chemical, mineral and amino acid composition of rapeseed meal with different origin[1,2,3,4,5]

Items		Canada	Australia [1]	China [2]	Europe [3]	Pakistan [4]	NRC´07	Feedstuffs´08	Bulgaria'12 [5]
									Chemical composition:
Crude Protein		36.0	37.30	37.0	34.0	40.10	33.90	38.0	36.35
Ether Extract		3.50	3.40	2.30	2.50	2.03	3.10	3.80	3.07
Fibre	Crude	11.7	9.90	12.1	12.4	12.80	9.70	11.1	13.7
	Acid Detergent	16.8	16.40	21.9	18.2	NA	16.80	NA	NA
	Neutral Detergent	20.7	24.10	35.1	28.1	NA	32.10	NA	NA
Ash		6.10	7.30	8.60	7.00	10.10	6.20	7.80	7.29
									Macroelements:
Calcium		0.62	0.56	0.71	0.76	NA	0.79	0.68	0.7
Phosphorus		1.06	0.96	1.04	1.13	NA	1.06	1.17	1.02
									Amino acids:
Lysine		2.00	2.02	1.64	1.86	1.86	1.85	2.02	4.73
Met + Cys		1.60	1.60	1.62	1.49	1.77	1.56	1.74	2.68

1 Spragg et al., 2007, 2,3 Feedbase, 2001, 4 Nadeem et al., 2005, 5 Yossifov et al., 2011a

Simultaneously, that reflected on diet digestibility, resp. animal performance. An important indicator for determining protein feeding value in ruminants diet is ruminal degradability (table 5). Degradability varied widely for DM (53.5 – 67.2 %) and CP (51.5 – 72.2 %) with highest values for our analyses.

Table 5. Comparative analysis of rapeseed meal (RSM) degradation in the rumen

Degradability (%):	Dry matter	Protein
Ha and Kennelly (1984)	57.40	67.00
Kirkpatrick and Kennelly (1987)	63.60	67.60
Kendall et al. (1991)	53.50	51.50
Cheng et al. (1993)	-	68.70
Piepenbrink and Schingoethe (1998)	65.10	53.10
Woods et al. (2003)	60.50	66.70
Yossifov (2013)*	67.15	72.19
Average:	61.21	63.83

* Unpublished data

Distillers' grains (DG-s). Distillers' grains are a co-product from the production of ethanol by dry (chemical) milling or wet method. Cereal starch from raw material is biotransformed into sugars, which are subsequently fermented into ethanol (*Katzen International Inc., 2011*). As a result of these processes is receiving main product (bioethanol) and co-product (in various forms). The litter is rich in nutrients (protein, fat, fiber, minerals and vitamins) that are triple higher versus raw material. The main bioethanol production is concentrated in the countries of North America and the main feedstock is corn. Qualities of the dried distillers' grains with solubles (DDGS) as a feedstuff in livestock diets are so high-valued that the import of this by-product is increased annually *(Shurson, 2006)*. Importing leaders within the EU are Ireland, Spain, Britain, Portugal, Netherland and Germany *(Shurson, 2006)*. Generated amounts of DDGS included by-products from the fermentation of corn, wheat and barley in dried form plus qualities fed in fresh (not dried) form *(Kyriazakis, 1992)*. Main producer of corn bioethanol worldwide is USA with produced DG co-products estimated up to 34.0 million tons. Short-term prognoses (2020) are augmentation with near 13 % (38.6 million tons) *(Hoffman et al., 2010)*. Soybean products annually loses new areas in USA as main oilseed crops replaced by area planted with corn intended for ethanol production (*Anon., 2011a*). The situation is different in Canada and the EU countries, where the main cereal energy crops used in bioetanol production are wheat followed by corn, rye, sorghum and cereal mix *(Piron et al., 2009; Paul et*

al., 2012). In some countries, such as Brazil and Australia as a raw material is used sugarcane *(Paul et al., 2012)*.

Statistic data on the produced, imported or fed quantities DDGS and RSM for the purposes of animal nutrition in our country lacking and it's difficult to be measured the problem. Simultaneously, lack the both data about amounts of co-products used in the feedproduction industry as a part of compound feed and fed alone in recent years. Till now, has been putted into operation seven large factories for bioethanol production. They're equipped with effective technological systems and realized the main product (bioethanol) and supporting co-products (DDG and DDGS). In a competitive market conditions over the years only two distillery manufactures has managed to escape. Their capacity is 10,000 tons/year. There are

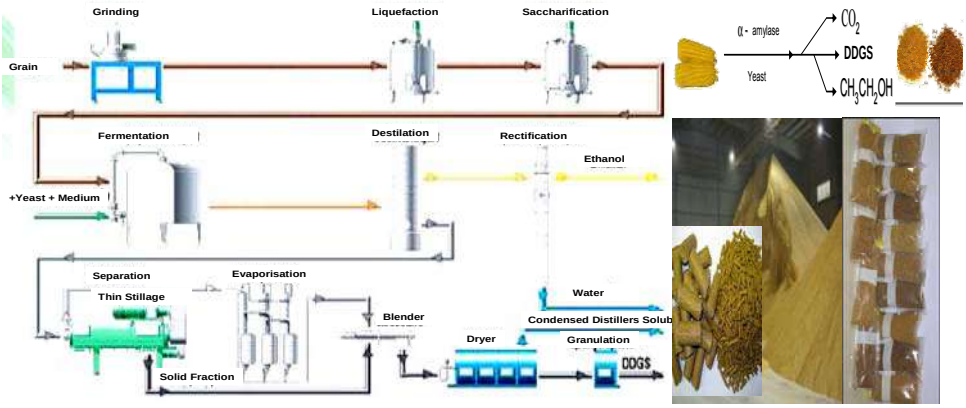

Figure 2. Technology of DDGS production

also a number of small distillery without significant market share. The sector problems are variable chemical composition of the final co-product, the lack of sufficient activity and enlightening knowledge. The technological processes could be summarized in the following scheme (figure 2). The lack of statistical data in the country about the produced and available quantities of DG co-products can be compensated by using the following equation (*CRFA, 2010*):

1 T DM from corn/wheat –> 387.0 L ethanol + 372.0 kg DDGS (330 kg DM) + 365.0 kg CO_2 + Q, [2]

Differences in technology processes (dry / wet milling) at this industry determined the variety of by-products. They could be summarized in the following classification (figure 3).

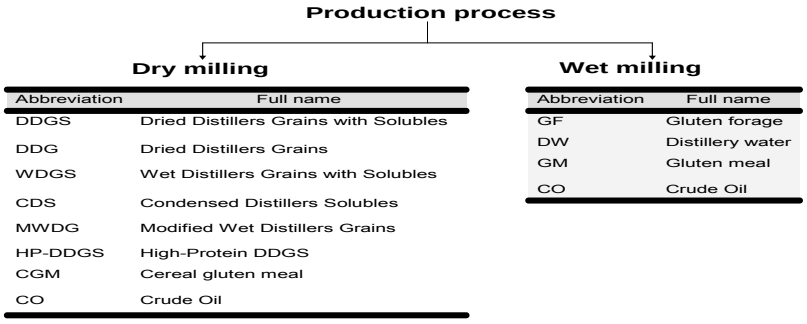

Figure 3. Classification of the by-products from bioethanol industry

Such heterogeneous group of products with relatively variable chemical composition applied their analysis to be compared difficult (table 6). Overviewed

Table 6. Comparative analysis of chemical and mineral composition of different bioethanol by-products[1,2]

Items	CDS	WDG	MWDG	DDG	DDGS	DDGSc	DDGSw
							Chemical composition:
DM	30-50	25-35	50	88-90	88-90	87.42	92.72
CP	15-25	30-35	30-35	25-35	25-32	26.40	36.85
EE	15-20	8-12	8-12	8-10	8-10	11.46	5.70
NDF	10-23	30-50	30-50	40-44	39-45	-	-
							Nutritive value:
DP	50	45-53	45-53	40-50	43-53	-	-
PDI	110-130	140-165	140-165	145-170	140-165	68.82	183.84
FUM/кg	1.7-2.0	1.6-1.9	1.6-1.9	1.5-1.8	1.55-1.8	1.07	1.13
							Macroelements:
Calcium	0.03-0.17	0.02-0.03	0.02-0.03	0.11-0.2	0.17-0.26	0.10	0.12
Phosphorus	1.3-1.45	0.5-0.8	0.5-0.8	0.41-0.8	0.78-1.08	0.93	0.97

1 Tjardes and Wright, 2002; 2 Yossifov and Kozelov., 2011 and 2012; 3 Yossifov et al., 2012; 4 Yossifov and Kozelov 2012a .

data about chemical, mineral composition and nutritive value of DG characterized these products as useful feed. CP content varied in wide range according to DM content (15 - 37 %). Our own data *(Yossifov and Kozelov, 2012)* showed highest CP level (36.9 %). Fat content also varied (5.7 - 20.0 %) and our data was in lower range (5.7 %). Protein feeding value of DDGS depends on its ruminal degradability (table 7). It varied widely (43.7 – 79.1 %) with highest values for our analyses.

Table 7. DDGS's protein degradability in rumen

Degradability (%):	Protein
Firkins et al. (1984)	46.0
Carvalho et al. (2005)	63.3
Kononoff et al. (2007)	57.0
Schingoethe et al. (2009)	47 - 64.0
Oba et al.(2010)	66.9
Kelzer et al. (2010)	43.7 - 66.9
Yossifov (2013)*	79.1
Average:	59.32

* Unpublished data

Characterization of biofuel by-products. Based on upper results, we form following advantages and disadvantages for analyzed biofuel by-products (table 8).

Table 8. Advantages and disadvantages of biofuel by-products

	Advantages	Disadvantages
Rapeseed meal (**RSM**)	High-quality protein feed with high biological value (AA)	High levels of sulfur
		Anti-nutritive factors (ANF-s)
	Constant chemical composition	Glycosinolates (tioglycosides);
	High palatability	Eruc acid
	Source of rumen undegradable protein (RUP)	Phenols – suppressing feed consumption and digestibility;
	Rich in PUFA	Phytates – suppressing phosphorus utilization
	Conjugated linoleic acid (CLA)	
	Low cost	Tannins – reducing the absorption of energy and protein, worsen meat color and flavor
	Applicability in our animal husbandry	
		Sinapins - suppressing consumption and gives a taste of the fish
Distillers Grains (**DG**)	Low cost	Maylard reaction
	Source of rumen degradable protein	Variable chemical composition
		High % Cu - sheep cuprotoxycosis
	Easy transport and storage	High % Ca - urinary calcules
	Nutrient concentration (x 3);	High % P - inorganic pollution
	Low-starch forage (ruminal acidosis	High % K - "wet litter";
		Mycotoxins
	Energy and/or protein source	
	Applicability in our animal husbandry	

Productive affects feeding by-products from biofuel industry in ruminant diets. The rate of biofuel by-products inclusion for optimal or maximal animal productivity is not unambiguous (table 9) among different species and

categories. Nowadays, searching maximal inclusion rate occupied a lot of nutritionists and animal researchers.

Table 9. Maximum inclusion levels of by-products from biofuel production, added to the diet of ruminants (%)

Species/Category	RSM	DG	DDG	WDG	CDS
Calves	No limit *	10 ***			
Lactating dairy cows		20 ***			
Beef		10 - 15 ***	10 - 20 ***	10 - 40 ***	10 ***
Heifer		10 – 40 ***			
Finishers		10 – 20 ***			
Fattening lambs	10 **	10 ***			
Lactating dairy ewes	10 **	10 ***			

*www.canolacouncil.org **www.countrywide.com ***www.ncga.com

DG-s are an excellent feedstuff for high-productive ruminant, ensured high level of animal performance (DMI, Milk yield, milk composition, etc.). Inclusion different levels DG (table 10) lead to different DMI, resp. milk yield and milk composition. One of the main reasons is concentration of nutrients (protein and energy) from cereal grains to DG by-products, which requires careful monitoring.

Table 10. Feeding DG in lactating dairy cows' diets*

Diet DG, % DM	*DMI*, кg.d^{-1}			*Milk yield*, кg.d^{-1}			*Milk fat*, %	*Milk protein*, %
	DDG	WDG	Total	DDG	WDG	Total	DG	DG
0	23.5^{c}	22.2^{B}	22.2^{B}	33.2	31.4	33.0	3.39	2.95^{a}
4 – 10	23.6^{BC}	23.7^{a}	23.7^{a}	33.5	34.0	33.4	3.43	2.96^{a}
10 – 20	23.9^{aB}	22.9^{aB}	23.4^{aB}	33.3	34.1	33.2	3.41	2.94^{a}
20 – 30	24.2^{a}	21.3^{aB}	22.8^{aB}	33.6	31.6	33.5	3.33	2.97^{a}
over 30	23.3^{BC}	18.6^{c}	20.9^{c}	32.2	31.6	32.2	3.47	2.82^{B}

* Kalscheur, 2005

Similar trends showed and RSM (table 11). Inclusion RSM in lactating dairy cow's diets significantly increased milk productivity (table 11). The main goal of RSM is its RUP concentration (table 5) affecting post-ruminal AA flow as a supplementation of microbial protein and AA.

Table 11. Feeding RSM in lactating dairy cows' diets (milk yield, кg.d^{-1})*

Study		Control	Experimental
Ingalls and Sharma	(1975)	23.0	23.7
Fisher and Walsh	(1976)	24.4	23.0
Vincent et al.	(1990)	25.1	26.7
Dewhurst et al.	(1999)	23.7	25.5
White et al.	(2004)	21.7	22.7
Maesoomi et al.	(2006)	27.0	28.0
Johansson and Nadeau	(2006)	35.4	38.4
Brito and Broderick	(2007)	40.0	41.1
Mulrooney et al.	(2008)	34.3	35.2
Average:		28.29	29.37

Data about feeding trials conducted with lactating dairy ewes are poor. DG and RSM are still unacceptable from farmers producing sheep milk. Their apprehensions are prompted by uncertain about profits (economical, etc.) of these forages *(Yossifov, 2012)*, especially at high-productive animals (table 12).

Table 12. Feeding RSM and DDGSw in lactating dairy ewes' diets*

GROUP / ITEMS		I /SFM/	II /RSM/	I I I /DDGSw/
Consumption (DMI), (kg) (%)		2.05 ± 0.10 (100 %)	2.05 ± 0.09 (100 %)	2.14 ± 0.10 (104 %)
Milk Yield (L) (%)	към 6.5 %	1.43 ± 0.21^{ab} (100 %)	1.47 ± 0.23^{ac} (103 %)	1.33 ± 0.18^{bc} (93 %)
Milk Protein, (%)		5.65±0.279	5.68±0.344	5.67±0.199
Milk Fats, (%)		7.10±0.801	7.26±1.169	7.02±0.540

aa - p<0.01 bb - p<0.001 cc - p<0.001 * Yossifov, 2012

Distillers' grains are often included in feedlot diets as protein and energy source with benefits at DMI, diet conversion and beef performance (table 13).

Table 13. Feeding DG in beef cattle' diets*:

ITEMS	% DM								
	DDGS					WDGS			
	0	20	30	40	50	0	20	30	40
Consumption (DMI), (кг.дн$^{-1}$)	10.12	10.20	10.20	9.90	9.44	10.17	10.53	10.56	10.49
Average Daily Gain (ADG), (кг)	1.57	1.74	1.76	1.73	1.66	1.56	1.69	1.70	1.66
DMI / ADG	6.45	5.95	5.81	5.74	5.71	6.58	6.29	6.45	6.58
DM feeding value	100	142	137	131	126	100	123	107	100

* Klopfenstein et al. (2008)

Impact of DG on beef performance aimed to minimized backgrounding costs with maximal deposition of muscle tissue and minimal – fats. At the same time meat quality from animals fed DG must be unaffected or with benefits.

As distinct from beef feedlot, lamb response to biofuel by-products supplementation is poor investigated. When evaluating *(Yossifov et al., 2012, Yossifov and Kozelov 2012a)* potential profits of DDGS and RSM for fattening lambs (table 14) was observed goal in all controlled parameters.

Table 14. Feeding DDGSc and RSM in fattening lambs' diets*

ITEM \ GROUP			SFM	RSM	DDGSc
Average live weight	Initial BW (IBW) (kg)		16.78±2.39	16.64±2.38	16.59±2.74
	(%)		(100%)	(99.17%)	(98.87%)
	Final BW (FBW) (kg)		31.58±5.39^{bb}	35.52±5.59^{b}	35.81±4.88^{b}
	(%)		(100%)	(105.46%)	(106.32%)
Average daily gain		(g)	180±0.6^{ab}	231±0.5^{b}	234±0.3^{a}
		(%)	100.00	120.42	123.04
Average daily consumption	TR	(kg)/h/d	1.29	1.26	1.35
		(%)	(100%)	(97.82%)	(104.45%)
	CP	(kg)/h/d	0.20	0.20	0.17
		(%)	(100%)	(98.83%)	(82.41%)
	FUG/h/d		1.35	1.37	1.14
		(%)	(100%)	(101.48%)	(84.44%)
Feed efficiency	TR/kg gain	(kg)	6.76	5.49	5.74
		(%)	(100%)	(81.23%)	(84.90%)
	CP/kg gain	(kg)	1.06	0.87	0.71
		(%)	(100%)	(82.07%)	(66.98%)
	FUG/ kg gain		7.07	5.96	4.85
		(%)	(100%)	(84.30%)	(68.60%)

aa - P<0.001, bb - P<0.01.

* Yossifov et al., 2012; Yossifov and Kozelov, 2012a

It's studied and effect of cereal origin of DDGS (table 15) with early (10 kg) weaned lambs *(Yossifov and Kozelov, 2012a).*

Table 15. Feeding DDGSw in fattening lambs' diets*

GROUP ITEM			SFM	DDGSw
Average live weight	Initial BW (IBW)	(kg)	10.73±3.23	10.57±2.99
		(%)	(100%)	(98.59%)
	Final BW (FBW)	(kg)	29.00±6.32^{c}	32.33±6.10^{c}
		(%)	(100%)	(111.48%)
Average daily gain		(g)	160±0.4^{c}	191±0.4^{a}
		(%)	100.00	119.38
Average daily consumption	DM	(kg)/h/d	0.83	0.84
		(%)	(100%)	(101.04%)
	PDI	(kg)/h/d	0.09	0.09
		(%)	(100%)	(98.62%)
	FUG/h/d		0.918	0.915
		(%)	(100%)	(99.67%)
Feed efficiency	PDI/kg gain	(g)	572.06	472.57
		(%)	(100%)	(83.13%)
	FUG/ kg gain		5.75	4.82
		(%)	(100%)	(83.83%)

aa - P<0.001, bb - P<0.01, cc – P<0.05. * Yossifov and Kozelov, 2012a

Conclusions

The continued growth of the biofuel industry result in an abundant supply of by-products that may be used as feedstuffs for ruminant. Increment of areas planted with energy crops promised enough qualities of rapeseed meal (RSM) and dried distiller's grains with solubles (DDGS). Their composition (chemical, mineral, amino acid (AA), etc.) supplied nutrients that ensured high productivity (gain, milk yield). Simultaneously, available essential nutrients improved quality of animal production (composition of meat and milk, etc.).

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Sporedni proizvodi u industriji biogoriva - alternativa tradicionalnim izvorima biljnih proteina u ishrani preživara

Lazar K. Kozelov, Marin R. Yossifov

Rezime

Proizvodnja biodizela i bioetanola je u stalnom porastu širom sveta. U tom smislu, povećava se i količina sporednih proizvoda. Cilj je bio da se procene i uporede glavni nusproizvodi iz industrije biogoriva (sačma uljane repice (rsm) i sušena destilovana džibra sa rastvorljivom materijom (ddgs)) kao izvora proteina biljnog porekla. Rad se zasniva i na pregledu literature i sopstvenih podataka. Ciljna vrsta domaćih živtonja su bili preživari različitih kategorija. Sprovedeno je nekoliko ogleda (laboratorija, balans i ishrana) sa sledećim pokazateljima: hemijski sastav, hraljiva vrednost, performanse životinja, itd. U celini, rezultati su pozitivno uticala i testirana krmiva se mogu koristiti kao alternativa konvencionalnim (sunc.sačma, sojina sačma, itd) proteinskim koncenttratima. Na osnovu ovih podataka smo došli do sledećih važnijih zaključaka:

- u obroku sa uljanom repicom (rsm) i ddgs, proizvedenim u bugarskoj, nije bilo značajne razlike u odnosu na ostale proizvedene širom sveta;
- obrok sa uljanom repicom koji sadrži minimalne koncentracije antinutritivnih faktora (glukozinolata - do 30 μmol.g-1) i eruka kiselina - do 2%) i može se preporučiti kao izvorproteina i / ili energije u obroku preživara bez ograničenja;
- preporučeno uključivanje 10% rsm u ishrani mlečnih ovaca u laktaciji je optimalno, i za tov jagnjadi;
- uključivanje ddgs u ishrani preživara bi moglo da bude i do 40 % .

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SLAUGHTER TRAITS, CARCASS COMPOSITION AND MEAT QUALITY OF CATTLE SLAUGHTERED AT DIFFERENT BODY WEIGHTS

M. Petričević, S. Aleksić, M. M. Petrović, N. Stanišić, M. Gogić, D. Nikšić, V. Petričević

Institute for Animal Husbandry, Autoput 16, 11080, Belgrade-Zemun, Republic of Serbia
Corresponding author: majanovakovic@live.com
Original scientific paper

Abstract: In this study, the influence of pre-slaughter body weight of domestic Spotted cattle on slaughter yield, carcass composition and important meat quality characteristics is presented. The cattle in the first group (G1) were slaughtered at average body weight of 531.9 kg, cattle from the second group (G2) at average body weight of 576.6 kg and cattle from the third group (G3) at average body weight of 615.4 kg. Based on the obtained results it can be concluded that the slaughter carcass traits of cattle did not differ significantly between the analysed groups. Body weight before slaughter had statistically significant effect on the share of bone in the leg/ham, the highest share was determined in the young cattle of group G1, and gradually decreased with increasing body weight. In terms of technological characteristics of *M. longissimus dorsi*, meat tenderness was statistically significantly lower in heavier cattle (G3) compared to lighter cattle (G2), while the lowest value for cooking loss of meat was determined for beef cattle in group G1 (31.90%).

Key words: cattle, domestic spotted cattle, dressing percentage, carcass composition, meat quality

Introduction

Fifty years ago the production and consumption of beef was dominant throughout the world. At that time 27 million tons of meat was produced and consumption was about 9 kg per capita per year. In recent years, production has doubled, but the consumption, due to the increase of population, remained the same. In recent decades significant increase in pork and especially in poultry production, compared to beef, was recorded. The main reason for this is the significantly lower price of pork and poultry meat due to their industrialized production. Beef is still considered the "queen of meat" and as such is the most

esteemed, and therefore the most expensive. This is probably the reason for the reduced consumption of beef compared to pork and poultry meat *(Čepin, 2007)*.

Weight gain, feed efficiency and carcass quality are of great economic importance to cattle producers. Variability of production traits may be contributed to differences in genetic origin, nutrition, slaughter and sex *(Mandell et al., 1997; Albertí et al., 2008)*. Satisfying the consumer's requirement for a consistent satisfactory product is the major target of beef producers and retailers. Meat quality is an important criterion that influences the decision of a consumer to purchase beef *(Sami et al., 2004)*. Meat quality is also dependent on many factors, such as primary carcass processing, cooling method and course of post mortem processes and tissue ratios that make up the basic composition of the meat.

Materials and Methods

The study was conducted on male cattle of domestic Spotted cattle. The experiment was performed on the experimental cattle farm, slaughterhouses and in laboratories of the Institute for Animal Husbandry (Belgrade, Serbia) on 90 heads of domestic Spotted cattle. During the fattening, the housing system was free, and all animals were fed the diet of same composition.

The cattle in the first group (G1, n=30) were slaughtered at average body weight of 531.9 kg, cattle from the second group (G2, n=30) at average body weight of 576.6 kg and cattle from the third group (G3, n=30) at average body weight of 615.4 kg. One day prior to slaughter cattle were deprived of food, but had free access to water. Animals were measured immediately before slaughter, after which they were slaughtered using standard techniques. After removing the skin and removing the head, front and rear legs and evisceration, carcasses were chilled at 4^0C for 24 hours, after which their weight was measured and cold carcass yield calculated with fat tissue and after the removal of fat tissue.

All left carcass sides were cut into major parts in accordance with the Rulebook on the meat quality of livestock for slaughter, poultry and game *(1985),* and the weight of the leg/ham, shoulder, back and loin were determined. The composition of tissues (muscle, fat, bone) was determined in the four major carcass parts. Chemical composition and technological properties of meat were determined in samples of *M. longissimus dorsi*. The following chemical parameters were determined: the water content by the method of drying of sample to constant weight at $102^{\pm}2^0$C *(SRPS ISO 1442, 1998)*, fat content by the method of extraction by Soxhlet with petroleum ether as solvent (*SRPS ISO 1444, 1998*), the amount of mineral matter (ash), by method of burning of the sample to constant weight at $550^{\pm}25^0$C *(SRPS ISO 936, 1999)* and nitrogen content by method of Kjeldahl (*SRPS ISO 937, 1992*). The following technological properties of meat were determined: the meat tenderness using the consistence-meter according to

Volodkevich *(Volodkevich 1938)* by cutting pieces of cooked meat crosswise to the direction of the muscle fibres, cooking loss, the mass difference of the meat before and after cooking at 100°C for 10 min, the total pigment content method of *Horsney (Bunning and Hamm, 1970)* and the pH value of meat 24 hours post-mortem using the pH meter with a combined electrode probe Hanna HI 83141 (Hanna Instruments, USA).

In order to determine the effect of pre-slaughter body weight on evaluated quality parameters of carcasses and meat, single factor experiment was performed using One way - ANOVA procedures of the program SPSS Statistics 20. If the effect of body weight showed statistically significance, t-test was performed to determine the actual statistical difference between the groups.

Results and Discussion

The influence of pre-slaughter body weight on slaughter results of studied cattle are shown in Table 1. Test results show that the highest dressing percentage/meat yield (57.11%) was determined in cattle of group G2 of average body weight of 576.6 kg, group G1 had dressing percentage/meat yield of 55.74% and 56.47% group G3. According to results obtained by *Aleksić et al. (2009)* for Domestic Spotted cattle of average pre-slaughter weight of 591.13 kg achieved carcass yield with kidney fat of 55.49% and yield without kidney fat was 54.63%. *Petričević et al. (2011)* reported that the share of kidney fat was 0.73% in cattle of average pre-slaughter weight of 500.0 kg and 0.81% in cattle of average pre-slaughter weight of 600.0 kg, which was confirmed by the results obtained in the present study.

Table 1. Dressing percentage/meat yield and share of kidney fat in cattle

Indicator	G1	G2	G3	t-test
Pre slaughter body weight (kg)	531.9^{a}	576.6^{b}	615.4^{c}	**
Cold carcass dressing percentage with fat (%)	55.74	57.11	56.47	ns
Cold carcass dressing percentage without fat (%)	55.03	56.33	55.67	ns
Kidney fat (%)	0.71	0.78	0.80	ns

ns – not significant
** significant at the level of ($p<0.01$)
a, b, c: values in the same column with different superscript are significantly different ($p<0.05$)

Share of carcass parts of the first I(leg/ham) and II category (shoulder, back and loin) did not differ significantly between groups of young cattle (Table 2). The same results were obtained by *Petričević et al. (2011).*

Table 2. The share of the main carcass side parts of beef carcasses

Carcass side parts (%)	G1	G2	G3	t-test
Leg/ham	28.23	29.16	29.77	ns
Shoulder	13.33	13.79	13.45	ns
Back	5.78	5.45	5.13	ns
Loin without beef steak	4.95	4.96	4.82	ns

ns – not significant

Data obtained by dissection of certain parts of carcass sides are shown in Table 3. Share of muscle tissue in the leg/ham was not statistically significantly different between the groups. *Waritthitham et al. (2010)* have reported that the proportion of muscle tissue decreases and the proportion of fat in the carcass increases in cattle of pre-laughter weight of 600 kg compared to cattle that were slaughtered at the weight of 500 kg. Similar results were obtained by *Sanudo et al. (2004)*. *Aleksić et al. (2007)* have reported that the proportion of muscle tissue in the leg/ham was around 86%, in carcass parts of the class II (shoulder) around 78% at the average cattle pre-slaughter weight of 597 kg.

Share of bone in leg/ham differed significantly ($p < 0.001$) and was higher in calves of group G2 (13.14%) compared to young bulls of group G3 (12.24%) *(D. Bureš and L. Bartoň, 2012)*. *Sanudo et al. (2004)* in their work report that the share of bones is lower in cattle slaughtered at the weight of 550 kg, compared with young bulls slaughtered at lower body weights.

Table 3. Share of muscle, fat tissue and bone in the main parts of beef carcass sides

Carcass part/tissue (%)	G1	G2	G3	t-test
Leg/ham				
Meat	82.56	81.15	80.76	ns
Fat	3.78	5.34	6.56	ns
Bone	13.32^{a}	13.14^{b}	12.24^{b}	***
Shoulder				
Meat	80.07	79.16	77.61	ns
Fat	2.74	4.02	6.02	ns
Bone	16.76	16.44	15.99	ns
Back				
Meat	70.13	71.81	71.71	ns
Fat	6.19	7.96	8.99	ns
Bone	21.87	18.93	17.88	ns
Loin				
Meat	71.26	70.80	70.26	ns
Fat	4.89	6.36	7.25	ns
Bone	23.05	22.72	20.87	ns

ns – not significant
*** significant at the level of ($p<0.001$)
a, b: values in the same column with different superscript are significantly different ($p<0.05$)

In terms of the basic chemical composition of *M. longissimus dorsi*, no statistically significant differences between the groups of young cattle were established. The minimum value of cooking loss was in the group G1 (31.90) and differed significantly from both groups ($p<0.001$). Meat tenderness was significantly better in group G3 compared to G2, where statistically significant differences were established ($p <0,05$). Similar results were also reported by *Sami et al. (2004)* and *Bureš and Bartoň (2012)*.

Table 4. Chemical composition and meat quality of *m. longissimus dorsi*

Item	G1	G2	G3	t- test
Water, %	75.62	75.66	75.29	ns
Fat, %	1.09	1.16	1.57	ns
Ash, %	1.10	1.09	1.07	ns
Protein %	22.18	22.07	22.05	ns
pH (24h)	5.69	5.59	5.63	ns
Cooking loss, %	31.90^{a}	38.74^{b}	37.10^{b}	***
Tenderness, %	10.67^{ab}	13.07^{a}	9.30^{b}	*
Total pigments, mg/kg	114.16	127.28	118.12	ns

ns – not significant
* significant at the level of ($p<0.05$)
*** significant at the level of ($p<0.001$)
a, b, c: values in the same column with different superscript are significantly different ($p<0.05$)

Conclusion

Based on the results obtained in this research it can be concluded that the carcass slaughter traits of cattle did not differ significantly between the analysed groups. Pre-slaughter body weight had a statistically significant effect on the share of bone tissue in the leg/ham, as it was determined that the group of young cattle G1 had the highest share of this tissue, which gradually decreased with increasing body weight.

In terms of technological characteristics of *M. longissimus dorsi*, meat tenderness was significantly lower in heavier cattle (G3) compared to lighter animals (G2), while the lowest value determined for meat cooking loss was in beef cattle group G1 (31.90%).

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Klanične karakteristike, sastav trupa i kvaliteta mesa junadi zaklanih pri različitim telesnim masama

M. Petričević, S. Aleksić, M. M. Petrović, N. Stanišić, M. Gogić, D. Nikšić, V. Petričević

Rezime

U radu je prikazan uticaj telesne mase pred klanje junadi domaće šarene rase na vrednosti randmana klanja, sastav trupa i važnije karakteristike kvaliteta mesa. Prva grupa junadi (G1) bila je zaklana pri dostizanju prosečne telesne mase od 531.9 kg, druga (G2) pri dostizanju prosečne telesne mase od 576.6 kg i treća (G3) pri dostizanju prosečne telesne mase od 615.4 kg. Na osnovu dobijenih rezultata može se zaključiti da se klanične karaketristike trupa junadi nisu statistički značajno razlikovale između analiziranih grupa. Telesna masa pred klanje imala je statistički značajan uticaj na udeo kostiju u butu, gde je utvrđeno da je kod junadi grupe G1 bio najveći i da se postepeno smanjivao sa porastom telesne mase. U pogledu tehnoloških osobina *M. longissimus dorsi*, mekoća mesa bila je statistički značajno manja kod težih junadi (G3) u odnosu na lakšu junad (G2), dok je najmanja vrednost kala kuvanja mesa utvrđena za junad grupe G1 (31.90%).

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THE EFFECT OF CROSSING SIMMENTAL CATTLE WITH FRENCH BEEF CATTLE BREEDS ON SLAUGHTER PROPERTIES AND QUALITY OF MEAT

M.M. Petrović*, S. Aleksić, D. Ostojić-Andrić, M.P. Petrović,V. Pantelić, N. Stanišić, M. Petričević

[1]Institute for Animal husbandry, Belgrade-Zemun, 11080 Zemun
* Corresponding author: Dr Milan M. Petrović, e-mail: milpet99@gmail.com
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Abstract: The study presents the review of the results and impact of crossing of Simmental breed with French fattening breeds on slaughter properties of meat and meat quality. The investigation of technological properties and sensory characteristics was conducted on a MLD sample of the region of 10th to 12th vertebra. Statistical and mathematical analysis was performed using the method of Least Squares mixed model with fixed effects (LS - Least Squares). The obtained results showed significant deviations of carcass weight and the amount of bones in carcass sides ($P < 0.05$) from the average. In crosses of F_1 generation obtained by crossing of Simmental cows with Limousine bulls higher meat yield by 11.5 kg was realized compared to the general average, and by 30.7 kg higher meat yield than in Simmental cattle. Highly significant deviation from the general average ($P<0.01$) was observed in slaughter yield (dressing percentage) and quantity of meat and fat in the carcass sides. Cross-section of MLD was the largest in meat of cattle (Simmental x Charolais). By using the method of sensory evaluation results were established for the important parameters of quality, softness/tenderness ("firmness" or texture) and succulence ("drieness") of cooked or roasted meat ($P<0.01$).

Keywords: beef, MLD, sensory properties, Simmental, Limousine, Charolais, technological quality

Introduction

Less than 35% of livestock production in total agricultural production (Serbia) indicates an under-developed agriculture of our country. Especially pronounced decline is in cattle population and thus the decline reflects on the production of products of animal origin (meat, milk). Therefore to make use of natural resources and to be able to be competitive in other markets, it is necessary

to look in livestock and cattle production for new models in meat production to bring about positive effects on the domestic market and export *(Jukna et al., 2009)*. Beef production in Serbia is mostly based on Simmental breed. The largest cattle breed in the Republic of Serbia is Simmental (about 85% of the population, or about 360.000 cows and heifers. Beef production for the last two decades in Serbia has decreased by more than 50.000 tons, according to *Petrović et al., (2011 and 2013)*. Crossing of different cattle breeds to obtain progeny that will exhibit improved properties with respect to the fattening and slaughter traits, have been used for decades in the World and Europe, as well as to achieve higher body mass for a shorter duration of fattening, greater daily weight gain and have better carcass characteristics of meat (heterosis effect). By applying the method of crossing in the offspring (F_1 generation) heterosis is manifested in fattening traits and meat quality traits. Factors contributing to the quality and quantity of meat are different and are of genetic and paragenetic nature (housing, rearing, care, food, etc.). The quality of meat is affect by the preparation for slaughter and post-mortem effects (slaughter, cooling of meat, etc..) Most authors have conducted research on the effects of crossing of different breeds of cattle on the yield and quality of beef - *Aleksić et al., (2006), Jukna Č. and Jukna V., (1998), Ostojić-Andrić et al., (2011), Maloney (2004), Pavlovski et al., (2004), Petrović et al., (2007)* and *Sazili et al., (1996).* Research results obtained by these groups of authors have indicated positive effects of crossing of several cattle breeds with French and other beef cattle breeds on the yield and quality of meat. Results of our research related to sensory and organoleptic properties of meat were generally consistent with those reported by other researchers indicating minor differences in the effect of the genetic structure on the mentioned properties *(Aleksić et al., 2011; Buchter, 1985; Harrington, 1985; Ostojić-Andrić et al., 2008).*

Material and Method

The present research included three groups of cattle of different genetic structure. The first group of slaughter cattle comprised the crosses of Simmental cows of lower production performance in regard to milk production with Charolais bulls (27 animals). The second group consisted of cattle, F_1 generation obtained by crossing of Simmental cows and bulls of French Limousine breed (23 animals). The third group of slaughter cattle were Simmental cattle (20 head). Young cattle obtained in our research were reared on experimental cattle farm of the Institute for Animal Husbandry, Belgrade-Zemun. Slaughter of animals and primary processing were carried out in the experimental slaughterhouse of the Institute and physical-chemical, thermal and other sensory testing in the laboratory of the Institute of Animal Husbandry. Upon completion of cooling, for 24 hours at 4^0C, dissection of

the three rib cut was performed. Three rib cut (region of the 9th-10th-11th rib) was always cut from the left chilled carcass side along the cranial line from 9 to 11 rib and cut paralel to the spinal column and vertical to the ribs. Investigation of physical-chemical properties and organoleptic properties was carried out on a sample of MLD, region of 10th to 12th vertebra. The evaluation was performed by three member score panel, using the score from 1 to 5, after reaching the harmonization of assessments and opinions.

Statistical and mathematical analysis was performed using the Least Squares Mixed Model with fixed effects (LS - Least Squares) using the Least-Squares Program (LSMLMW), Harvey, in 1990. When analyzing the impact of genetic groups of cattle on meat quality traits, the following model was used:

$$Y_{ij} = \mu + GG_l + b_1(x_1-x_2) + e_{ij}$$

Where:

μ = general average,
GG_l = fixed effect of genetic group (1-3),
b_1 = linear regression effect of pre-slaughter body weight,
e_{ij} = random error

Results and Discussion

Statistical analyzes showed significant deviations of carcass weight and the amount of bone in the carcass ($P<0.05$), from the general average. In F_1 generation crosses obtained by crossing of Simmental cows with Limousine bulls, the higher meat yield was realized by 11.5 kg than the general average, and by 30.7 kg than in Simmental cattle (Table 1).

Table 1. Slaughter properties of cattle, the mean values (LSM), the error of mean values (SE) and the deviation from the general average ($\hat{c}_i$)

Traits	LSM	S.E.	Sim x Cha ($\hat{c}_i$)	Sim x Lim ($\hat{c}_i$)	Simm ($\hat{c}_i$)	$F_{exp.}$
Carcass weight with fat, kg	399.9	2.1	7.7	11.5	-19.2	*
Killing out, %	60.0	0.5	1.7	2.1	-3.8	**
Kidney fat, kg	5.1	0.3	-0.8	0.7	0.1	NS
Carcass side meat, kg	141.0	1.8	9.1	5.3	-14.4	**
Carcass side bones, kg	27.5	0.9	-1.0	-0.7	1.7	*
Carcass side fat, kg	6.1	0.3	-0.9	-0.6	1.5	**

NS NOT significant ($P>0.05$), * Significant ($P<0.05$), ** Highly Significant ($P<0.01$)
Sim x Cha - F_1 generation between Simmental and Charolais beef cattle
Sim x Lim - F_1 generation between Simmental and Limousine beef cattle
Simm - F_1 generation between Simmental and Limousine beef cattle

Highly significant deviations from the general average (P<0.01) were observed in regard to slaughter yield trait and quantity of meat and fat in the carcass side. Thus, the carcass yield of Simmental cattle was lower by 3.8% than the general average and by 5.9% lower than in crossbred cattle (Limousin x Simmental). Animals, crosses of Simmental x Charolais had more meat in the carcass sides by 9.1 kg and 23.5 kg more than the Simmental breed (Table 1).

The influence of the genetic structure on traits of MLD as LSM deviations ($\hat{c}_i$) is shown in Table 2. Results obtained for MLD properties revealed statistically significant deviations from the general average in terms of colour and pH_{24} value of MLD (P<0.05). Colour of meat of cattle - crosses with the French beef cattle breeds was brighter compared to red meat of MLD from Simmental breed. For properties of marbling and muscle structure of MLD no significant differences (P>0.05) were established.

Table 2. MLD# and pH_{24} value and physical characteristics of meat

Traits	LSM	S.E.	Sim x Cha ($\hat{c}_i$)	Sim x Lim ($\hat{c}_i$)	Simm ($\hat{c}_i$)	$F_{exp.}$
Colour (1-5)	4.6	0.09	0.31	0.42	-0.73	*
Marbling (1-5)	4.5	0.07	-0.27	0.18	0.09	NS
Structure estimate.(1-5)	4.5	0.07	0.29	0.49	-0.78	NS
pH_{24} value	5.9	0.05	-0.09	-0.10	0.19	*
Physical characteristics of meat						
Firmness (Voloktiewitsch)	5.8	.08	-0.9	-1.0	1.8	**
Muscle fibre diameter, μm	45.9	.09	-1.4	-1.8	3.2	**
Total pigment, ppm	85.9	1.1	0.5	3.3	-3.8	*
Muscle eye MLD, cm^2	104.9	.71	2.7	-1.0	-1.7	**

NS NOT significant (P>0.05), * Significant (P<0.05), ** Highly Significant (P<0.01)
MLD# - musculus longissimus dorsi

Statistically significant deviations (P <0.01) of physical characteristics of meat (meat firmness, thickness of muscle fiber and cross-sectional area of MLD) were established. The total meat pigment showed significant variation (P <0.05). Cross-section of MLD was the largest in meat of cattle Simmental x Charolais.

Table 3. Organoleptic traits

Traits	LSM	S.E.	Sim x Cha ($\hat{c}_i$)	Sim x Lim ($\hat{c}_i$)	Sim ($\hat{c}_i$)	$F_{exp.}$
Organoleptic traits						
Cooking test - firmness (1-5)	4.5	0.09	0.39	0.41	-0.91	**
Cooking test – dryness (1-5)	3.7	0.09	0.33	0.39	-0.72	**
Cooking test – taste (1-5)	4.1	0.07	0.09	0.06	-0.15	**
Cooking test – aroma (1-5)	4.2	0.08	-0.10	0.19	-0.09	NS
Roasting test – firmness	4.5	0.06	0.12	0.29	-0.41	*
Roasting test – dryness	3.6	0.07	0.08	0.09	-0.17	*
Roasting test – taste	4.0	0.09	-0.11	0.20	-0.09	*
Roasting test – aroma	4.1	0.07	-0.10	0.05	0.05	NS

NS NOT significant (P>0.05), * Significant (P<0.05), ** Highly significant (P<0.01)

The results of the impact of genetic structure of cattle on organoleptic properties are shown in Table 3. By using the method of sensory evaluation results were obtained for the important parameters of meat quality, softness/tenderness ("firmness" or texture) and succulence ("dryness") of cooked or roasted meat (P<0.01).

Conclusion

The results of the present study showed that in the statistical analyzes, significant deviations of carcass weight and the amount of bones in carcass sides (P<0.05) from the average were established. In F_1 generation crosses obtained by crossing of Simmental cows with Limousine bulls, the higher meat yield was realized by 11.5 kg than the general average, and by 30.7 kg than in Simmental cattle.

Highly significant deviations from the general average (P<0.01), were observed for slaughter yield trait and quantity of meat and fat in the carcass sides. Results obtained for MLD properties revealed statistically significant deviations from the general average in terms of colour and pH_{24} value of MLD (P<0.05). Colour of meat of cattle - cross with the French beef cattle breeds was brighter than red meat of MLD from Simmental cattle. For properties of marbling and muscle structure of MLD no significant differences (P>0.05) were established.

Statistically significant deviations (P <0.01) of physical characteristics of meat (meat firmness, thickness of muscle fiber and cross-sectional area of MLD) were established. Cross-section of MLD was the largest in meat of cattle Simmental x Charolais. By using the method of sensory evaluation results were obtained for the important parameters of meat quality, softness/tenderness

("firmness" or texture) and succulence ("dryness") of cooked or roasted meat ($P<0.01$).

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Efekat ukrštanja simentalske sa francuskim tovnim rasama goveda na klanične karakteristike i kvalitet mesa

M.M. Petrović, S. Aleksić, D. Ostojić-Andrić, M.P. Petrović,V. Pantelić, N. Stanišić, M. Petričević

Rezime

Manje od 35% stočarstva u ukupnoj poljoprivrednoj proizvodnji (Srbija) ukazuje na nedovoljno razvijenu poljoprivredu naše zemlje. Posebno je izražen pad broja grla goveda a samim tim i proizvodnje produkata animalnog porekla (meso,mleko). Proizvodnja junećeg mesa u Srbiji se uglavnom zasniva na simentalskoj rasi. Proizvodnja junećeg mesa je za dve zadnje decenije u Srbiji smanjena za više od 50.000t. Primenom metoda ukrštanja kod potomaka (F_1 generacija) se ispoljava efekat heterozisa kako osobina tovnosti tako i svojstava kvaliteta mesa. Faktori koji doprinose količini i kvalitetu mesa su različiti i odnose se na genetske i paragenetske (odgoj,nega,ishrana i dr). Na kvalitet mesa utiče priprema za klanje, i postmortalni uticaji (klanje, postupak hlađenja mesa i dr.).

Naša istraživanja su obuhvatila tri grupe junadi različite genetske strukture. Prva grupa junadi za klanje je dobijena iz ukrštanja simentalske rase goveda nižih proizvodnih sposobnosti u pogledu proizvodnje mleka i bikova šarole rase. Druga grupa je obuhvatila junad, meleze F1 generacije dobijene ukrštanjem krava simentalske rase i bikova francuske limuzin rase. Treća grupa junadi za klanje su bila grla simentalske rase. Junad obuhvaćena našim istraživanjem su dobijena i gajena na ekperimentalnoj farmi goveda, Instituta za stočarstvo,Beograd-Zemun. Klanje grla i primarna prerada su obavljeni u kapacitetu ekperimentalne klanice Instituta a fizičko-hemijska, senzorna termička i druga ispitivanja u laboratoriji Instituta za stočarstvo. Po završenom hlađenju, tokom 24 na 4^0C, obavljena je disekcija trorebarnog kotleta. Trorebarni kotlet (regija 9-10-11 rebro) je uvek odsecan od leve ohlađene polutke duž kranijalne linije od 9 do 11. rebra i paralernim rezom u odnosu na kičmeni stub i vertikalnim rezom u odnosu na rebra.

Ispitivanje fiziko-hemijskih osobina i organoleptickih osobina obavljeno je na uzorku MLD-a regija 10. do 12. kičmenog prsljena. Ocenjivanja su obavili trojica ocenjivača ocenom od 1-5 nakon usaglašavanja ocena i mišljenja. Statističko-matematička analiza obavljena je primenom mešovitog modela najmanjih kvadrata sa fiksnim uticajima (LS - Least Squares).

Statistički analizirano, ustanovljena su značajna odstupanja mase trupa i količine kosti u u polutkama ($P<0.05$) od opšteg proseka. Kod meleza F_1 generacije dobijenih ukrštanjem simentalskih krava sa limozin bikovima je ostvaren prinos mesa za 11.5 kg više u odnosu na opšti prosek, odnosno 30.7 kg više u odnosu na junad simentalske rase.

Visoko značajna odstupanja od opšteg proseka($P<0.01$) su ustanovljena kod osobina randmana klanja i količine mesa i loja u polutkama. Tako je randman klanja kod junadi simentalske rase za 3.8% manji u odnosu na opšti prosek i 5.9% manji u odnosu na grla meleze (simmental x limousin). Grla, melezi (simmental x sharolais) su imali više mesa u polutkama za 9.1 kg odnosno 23.5 kg više u odnosu na količinu mesa junadi simentalske rase.

Uticaj genetske strukture na osobine MLD kao LSM odstupanja (ĉi) prikazan je u tabeli 2. Rezultati osobina MLD ukazuju na statistički analizirano značajna odstupanja od opšteg proseka u pogledu boje i vrednosti pH24 value MLD ($P<0.05$). Boja mesa grla junadi dobijenih iz ukrštanja sa francuskim tovnim rasama je svetlija u odnosu na crveno meso MLD junadi simentalske rase. Za osobine mramorinosti i strukturu mišića MLD nisu ustanovljene značajne razlike ($P>0.05$). Ustanovljena su statistički visoko značajna odstupanja ($P<0.01$) fizičkih karakteristika mesa (tvrdoće mesa, debljine mišićnog vlakna i površine poprečnog preseka MLD). Ukupni pigment mesa je pokazao značajna variranja ($P<0.05$).

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VARIABILITY OF MILK TRAITS IN THE POPULATION OF SIMMENTAL CATTLE IN SERBIA

D. Nikšić[1*], M.M. Petrović[1], V. Pantelić[1], D. Ostojić-Andrić[1], V. Caro-Petrović[1], P. Perišić[2], M.D Petrović[3]

[1] Institute for Animal Husbandry, Belgrade-Zemun, 11080 Zemun
[2] University of Belgrade, Faculty of Agriculture, Belgrade-Zemun, 11080 Zemun
[3] Faculty of Agronomy, Čačak, 32000 Čačak
*Corresponding author: Dragan Nikšić, e-mail: draganniksic84@gmail.com
Original scientific paper

Abstract: The work is the presentation of performance data and phenotypes of milk traits of Simmental cows in Serbia. The analysis of quantitative phenotypic parameters was carried out in four breeding areas and on dozen of large and small farms housing and rearing 1370 cows – first calving heifers. The obtained data were analysed using the method of Least Squares and analysis results were obtained by applying mathematical-statistical data analysis using mixed models. Mathematical - statistical analysis of data was performed using linear methods with fixed effects, using the method of Least Squares (LS method). Based on these results, it was established that the breeding area, statistically analysed, caused highly significant deviations ($P < 0.01$) of production traits from the general population average. Calving season has influenced significantly ($P<0.05$), and the interaction of area and calving season has produced a highly significant variation in milk yield, milk fat and protein ($P < 0.01$).

Key words: cow, phenotype, milk, milk fat, protein**.**

Introduction

The most numerous breed of cattle in the Republic of Serbia is Simmental breed. At the beginning of year 2013, preliminary results of the agricultural census in the Republic of Serbia, performed in 2012, were obtained. According to the census results, in Serbia there is a total of 908.990 heads of cattle, of which about 450.000 cows and pregnant heifers. In regard to the breed structure of cattle in Serbia, it is estimated that the Simmental breed makes up about 85%, or around 360.000 cows and heifers *(Perišić et al., 2009 and Petrović M.M. et al., 2013)*. Simmental cattle are increasingly reared in intensive farming systems, and on increasing number of farms in Serbia, with over 50 heads of breeding females *(Bogdanović et al., 2012)*.

In some areas of Serbia is semi-intensive rearing of cattle in herds of several cows is still present. Genetic improvement of the breed is mainly focused on breeding and selection in purebred *(Petrović, M.M. et al., 2009)*. Improving the genetic basis of the population of Simmental cattle in our conditions is mainly done through quality bulls (Germany, Austria and others.) And in recent years there is a number of imported heifers of high-quality genetic from the best European populations of this breed.

Improving the phenotypes of milk performance requires continuous work, which includes the systematic improvement of quantitative genetic traits and work on their improved expression/demonstration. High yield of milk, milk fat and protein, in addition to the selection, requires and optimal provision of para-genetic factors (nutrition, housing, rearing, health care, etc.). Studies of quantitative characteristics and performance data of this breed of cattle and the influence factors on para-genetic same properties have been presented by following authors: *Miesenberger and Fuerst (2006); Pantelić, V., (2006; 2010)., Petrović, M. M. et al. (2006; 2009), Gerber et al. (2008), Nikšić et al. (2011), Petrović M.D. et al. (2012).*

Material and Methods

Data included in the analysis were collected in four breeding areas (regions) in Serbia. These areas are distinguished by geographical features (altitude, precipitation, soil quality, duration of growing season, etc.). Also there were differences in the genetic quality of cattle, the method and the quality of food and nutrition management in production, etc. The research included 1370 cows – first calving heifers of Simmental breed that have calved, and concluded lactations in 2011 and 2012. First calving season includes the months of February, March and April, the second, May, June and July, the third, August, September and October, and the fourth, November, December and January.

The bull-sires were of Simmental breed and come from local AI centres and from abroad. Cows – first calving heifers were kept loose or tied, depending on the farm. Animals were fed usual diets depending on the degree of intensity of production and on breeding areas, and there were also differences in the quality and quantity of forage, i.e. amount of concentrated feed and roughage.

Statistical - mathematical analysis was performed using the Least Squares Mixed Model with fixed and random effects (LS - Least Squares and BLUP - Best Linear Unbiased Prediction) program using the Least Squares Program (LSMLMW), Harvey, 1990. When analysing the influence of region, year and season of calving on dairy cows in first lactation, the following model was used:

$$Y_{ijkl} = \mu + OP_l + G_j + S_k + GS_l + b_1(x_1-x_2) + e_{ijklm}$$

Where:

Yijkl – individual animal (*m*) reared in area (*i*), calved in year (*j*) and season (*k*),
μ = general population average,
OP_l = fixed effect of breeding area (1-4),
G_j = fixed effect of year of calving (1-2),
S_k = fixed effect of the season (1-4),
OPS_l = fixed effect of the breeding area/calving season interaction,
b_1 = linear regression effect of age at calving,
e_{ijklm} = random error

Results and Discussion

First calving heifers our research produced 4913 kg milk with 193.1 kg of milk fat and 3.93% of milk fat. The total amount of protein in milk produced in the first lactation was 161.1 kg with 3.29% protein (Table 1).

Cows reared in area 2 are produced 508.9 kg more milk compared to the general average and 900 kg more than those reared in the breeding area 4.

Table 1. Effect of systematic factors as LSM deviation ($\hat{c}_i$) on productive traits

Traits	Milk, kg		Milk fat, kg		Milk fat,%		Milk protein, kg		Milk protein,%	
M	4913		193.1		3.93		161.1		3.29	
S.E.	168.8		6.8		.03		5.9		.03	
Breeding Area	$\hat{c}_i$	LSM	$\hat{c}_i$	LSM	$\hat{c}_i$	LSM	$\hat{c}_i$	LSM	$\hat{c}_i$	LSM
1	69.6	4978	-1.6	193.6	0.1	3.89	-2.7	163.8	0.1	3.29
2	508.9	5422	-8.9	202.8	0.2	3.74	-12.4	173.5	0.1	3.20
3	-188.1	4725	2.7	190.4	-0.1	4.03	2.6	159.2	-0.1	3.37
4	-392.0	4521	8.1	184.0	-0.2	4.07	11.9	149.6	-0.1	3.31
Season of Calving	$\hat{c}_i$	LSM	$\hat{c}_i$	LSM	$\hat{c}_i$	LSM	$\hat{c}_i$	LSM	$\hat{c}_i$	LSM
I	206,6	5120	-5.1	198.1	0.1	3.87	-13.4	176.1	-0.1	3.44
II	-98.1	4814	10.9	180.1	0.1	3.75	1.4	161.0	-0.1	3.35
III	-207.1	4706	5.70	186.0	-0.1	3.96	12.0	149.1	01	3.17
IV	97.3	5010	-11.1	204.1	-0.1	4.08	-0.5	162.1	0.1	3.25

Statistically, breeding area influenced highly significant ($P<0.01$), the deviations of milk yield, milk fat and milk protein yield (Table 2).

Season of the beginning of lactation in cows - first calving heifers, i.e. calving season showed significant deviations in regard to yields of milk, milk fat and milk protein in relation to the general average (P<0.05). So heifers which had calved in February, March and April produced 206 kg of milk more than the general average, or 413 kg more milk than those whose lactation beginning was in August, September and October (Table 1)

Table 2. Least – Squares analysis of variance (exp. F)

Influences	Milk, kg	Milk fat, kg	Milk fat,%	Milk protein, kg	Milk Protein,%
Breeding Area	11.475**	11.879**	25.248**	10.654**	23.876**
Season of calving	2.801*	3.643*	3.565*	2.987*	2.876*
Breeding area/season of calving	2.818**	2.889**	1.867^{NS}	2.111**	1.939^{NS}

NS – P>0.05 * – P<0.05 ** – P<0.01

First calving heifers in the present study produced 4913 kg of milk with 193.1 kg of milk fat and 3.93% of milk fat and 161.1 kg and 3.29% of milk protein. The results obtained in our research were significantly higher compared to the results of the milk performance of the same breed animals obtained by *Pantelić (2006; 2010), Petrović, M. M. et al., (2006; 2009)* and *Petrović M.D. et al. (2012).* The results of the present study are similar to those obtained in Bavaria for the same breed by *Gerber et al. (2008).* The results obtained in our study were slightly higher compared to those established by *Nikšić et al. (2011). Gerber et al. (2008)* has found differences in the production of milk fat and protein, which ranged from 178.1 to 267.9 kg and 146.1 kg to 227.5 kg, respectively, depending on the housing system (the extensive and intensive). The results obtained in our research showed a positive genetic trend in regard to quantitative traits of milk performance of domestic Simmental cows. Comparing our (last presented) results and the results of the above-mentioned authors it can be concluded that the milk yield of Simmental cows (main/basic herd) annually in Serbia increases by at least 100 kg of milk per cow (depending on genetics, area, feeding technology and housing).

Conclusion

In the breed structure of cattle in Serbia, it is estimated that the Simmental breed makes up about 85%, or around 360.000 cows and heifers. Simmental cattle are increasingly grown in intensive farming systems and on increasing number of farms in Serbia, with over 50 heads of cows.

Genetic improvement of the breed is mainly focused on breeding and selection in pure breed. Improving the genetic basis of the population of Simmental

cattle in our conditions is mainly done through quality bulls (Germany, Austria and others.) and in recent years there is a number of imported cattle of high-quality genetic from the best European populations of this bred.

Improving the phenotypes of milk performance requires continuous work, which includes the systematic improvement of quantitative genetic traits and work on their improved expression/demonstration. High yield of milk, milk fat and protein, in addition to the selection, requires and optimal provision of para-genetic factors (nutrition, housing, rearing, health care, etc.). Being that the fastest improvement through selection and genetic improvement of production traits is through choice of bull sires this should be given the utmost attention. Knowledge of the influence of environment factors on quantitative traits is very important with respect to their importance in achieving breeding goals and good economic results.

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Varijabilnost osobina mlečnosti u populaciji simentalskih goveda u Srbiji

D. Nikšić, M.M. Petrović, V. Pantelić, D. Ostojić-Andrić, V. Caro-Petrović, P. Perišić, M.D Petrović

Rezime

Najbrojnija rasa goveda u Republici Srbiji je simentalska. Početkom 2013.godine dobijeni su preliminarni rezultati popisa poljoprivrede u Republici Srbiji, obavljenog 2012.godine. Prema rezultatima popisa u Srbiji ima ukupno 908.990 grla goveda od čega je oko 450000 krava i steonih junica. U rasnoj strukturi goveda u Srbiji, procenjuje se da simentalska rasa čini oko 85%, odnosno oko 360000 krava i junica. Simentalska goveda se sve više gaje u intezivnim uslovima držanja, nege i ishrane, odnosno sve je veći broj farmi u Srbiji sa preko 50 grla plotkinja. U nekim područijima Srbije je to još uvek poluintezivno držanje u stadima od nekoliko grla krava. Genetsko unapređenje ove rase se uglavnom sprovodi odgajivanjem i selekcijom u čistoj rasi. Unapređenje genetske osnove populacije simentalskih goveda u našim uslovima se uglavnom obavlja preko kvalitetnih bikova (Nemačka, Austrija i dr.) a u poslednjim godinama nije zanemarljiv broj uveženih genetsko visoko kvalitetnih junica iz najboljih evropskih populacija ove rase. Unapređenje fenotipova mlečnosti zahteva kontinuiran rad koji obuhvata sistematsko poboljšanje kvantitavnih genetskih osobina i permanentni rad

na njihovom poboljšanom ispoljavanju. Visok prinos mleka, mlečne masti i proteina, pored selekcije zahteva i optimalno obezbeđenje paragenetskih faktora (ishrana, držanje, odgoj, nega i dr.).

Podaci obuhvaćeni analizom su prikupljeni u četiri odgajivačka područja (regiona) u Srbiji. Ova područja se razlikuju po geografskim karakteristikama (nadmorska visina, količina padavina, kvalitet zemljišta, trajanje vegetacije i dr.). Takođe razlike su u genetskom kvalitetu grla, načinu i kvalitetu hrane i ishrane, menadžmentu u proizvodnji i dr. Istraživanjem je obuvaćeno 1370 grla krava-prvotelki simentalske rase koje su se otelile, odnosno zaključile lataciju 2011 i 2012 godine. Prvom sezonom telenja su obuhvaćeni meseci februar, mart i april., drugom, maj, jun i jul., trećom, avgust, septembar i oktobar., četvrtom, novembar, decembar i januar. Bikovi-očevi su simentalske rase i potiču iz domaćih VO centara i iz inostranstva. Prvotelke-krave su držane slobodno ili vezano u zavisnosti od farme. Grla su hranjena uobičajeno u zavisnosti od inezivnosti proizvodnje i područja gajenja a razlike su bile u kvalitetu hraniva odnosno količini kabaste i koncentovane hrane.

Statističko matematička analiza obavljena je primenom mešovitog modela najmanjih kvadrata sa fiksnim i random uticajima (LS - Least Squares i BLUP - Best linear Unbiased Prediction) primenom programa najmanjih kvadrata (LSMLMW), Harvey,1990.

Prvotelke obuhvaćene našim istraživanjima su proizvele 4913 kg mleka sa 193.1kg mlečne masti i 3.93% mlečne masti. Ukupna količina proteina u mleku, proizvedena u prvoj laktaciji je iznosila 161.1 kg sa 3.29% proteina. Krave gajene u području 2 su proizvele 508.9 kg mleka u odnosu na opšti prosek a 900 kg mleka više od onih koje su gajene u odgajivačkom području 4. Statički analizirano, odgajivačko područje je visoko značajno prouzrokovalo ($P<0.01$) odstupanje prinosa mleka, mlečne masti i proteina. Godišnje doba početka laktacije krava – prvotelki odnosno sezona telenja pokazuje značajna odstupanja prinosa mleka, mlečne masti i proteina u odnosu na opšti prosek ($P<0.05$). Tako su junice oteljene u februaru, martu i aprilu proizvele 206 kg mleka više u odnosu na opšti prosek, odnosno 413 kg mleka više u odnosu na one čiji je početak laktacije bio u avgustu, septembru i oktobru.

Poredeći naše (poslednje iznete) rezultate i rezultate napred navedenih autora može se zaključiti da se prinos mleka simentalskih krava (matični zapat) u Srbiji godišnje uvećava za najmanje 100 kg mleka po kravi (zavisno od genetike, područja , odnosno tehnologija ishrane i držanja).

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STUDY OF THE VARIABILITY OF MILK TRAITS IN THE POPULATION OF HOLSTEIN FRIESIAN CATTLE IN CENTRAL SERBIA

M. Lazarević[1*], M. M. Petrović[1],V. Pantelić[1], D. Ružić-Muslić[1], V. Bogdanović[2], R. Đedović[2], M. D. Petrović[3]

[1] Institute for Animal Husbandry, Belgrade-Zemun, 11080 Zemun
[2] University of Belgrade, Faculty of Agriculture, Belgrade-Zemun, 11080 Zemun
[3] Faculty of Agronomy, 32000 Čačak
*Corresponding author: Marina Lazarević, e-mail: marinaplazarevic@gmail.com
Original scientific paper

Abstract: The work includes the review of production results, i.e. milk performance phenotypes of Black and White Holstein cows in Central Serbia. The analysis of quantitative phenotypic parameters was carried out in five regions (breeding regions) on several large and small farms which housed 1090 cows - first calving heifers.Using the method of Least Squares - the fixed model, highly significant deviation of milk yield, milk fat from the average ($P < 0.01$) was established. Cows reared in the region 4 in the first lactation produced 503 kg less milk compared to the general average and 852 kg less milk than those reared in the region 3. Birth year caused statistically significant differences in milk yield and milk fat yield ($P < 0.05$), except % of milk protein where no significant variations ($P > 0.05$) were determined. Season in which the cows had calved caused a highly significant deviation of milk yield ($P < 0.01$) and significant deviations of yields of milk fat and protein ($P < 0,05$).

Keywords: Holstein, cow, phenotype, milk, milk fat, protein

Introduction

Black and white Holstein Friesian cattle breed is the prevailing breed of dairy cattle in the world (about 90% of the total milk production). Holstein cattle are bred in Europe, mainly in the lower regions. In the Republic of Serbia around 100.000 cows and heifers of Holstein-Friesian breed are grown *(Petrović et al., 2013)*. The majority of them are reared in Vojvodina, a small number is grown in central Serbia. With the exception of cows that are reared in the vicinity of Belgrade organized in a very intense production of several large farms (PKB Padinska Skela, PIK Zemun, BD Agro Dobanovci, etc.), a number that is grown on

small farms in Central Serbia is small and amounts to a few thousand heads (under the milk recording control less than 3000). That is why our research was conducted and the goal was to determine how often in semi-intensive conditions of care and nutrition, quantitative genetic potential of milk production traits of the breed is exerted. In recent years (last decade) began breeding of this breed of dairy cattle on small farms in Central Serbia. Traditionally, farmers in these regions are directed towards the production of milk and meat from Domestic Spotted and Simmental cattle that are less demanding in terms of nutrition, rearing and that give excellent results in less intensive conditions (milk, meat). Market, competition, intensifying and specialization in manufacturing led to the need for growing od dairy breeds of cattle with improved milk performance and increasingly in these parts of Serbia farms are formed with higher number of cattle (15-50).

In addition to feeding and care, particular attention should be paid to the genetic improvement of cattle. Genetic improvement of Black and White cattle in Serbia is achieved by selection and breeding of Holstein - Friesian breed. Methods of genetic improvement are essentially based on the use of high-quality bulls of Holstein breed from domestic artificial insemination (AI) centers and the use of imported seed. Many of the domestic and foreign researchers and scientists are involved and are working on the improvement of milk production phenotypes that require continuous operation and include the systematic improvement of quantitative genetic traits and continuous work on their improved expression. High yield of milk, milk fat and protein, in addition to the selection measures requires providing of appropriate paragenetic factors (nutrition, housing, rearing, health care, etc.). The following authors have studied quantitative characteristics and performance data for this breed of cattle and the influence of paragenetic factors on same properties: *Gaidarsca et al. (2004), De Marchi et al. (2008), Pantelić et al. (2010; 2011), Pirlo et al. (2000), Petrović et al. (1999a;1999b), Kuczyńska et al. (2012)* and *Stojić et al. (2011).*

Material and Methods

Data included in the analysis were collected in five regions (breeding regions) in Central Serbia. The regions differ in terms of geographical characteristics and hence the production of feed for cattle feeding. The differences are in the method and the quality of the nutrition, management of production, the genetic quality of cattle, etc. The research included 1090 cows-first calving heifers of Black and White Holstein Friesian breed. These animals have calved, i.e. concluded the lactation in the period from 2010 to 2013. Calving years of these animals were divided into four seasons: I (February, March and April), II (May,

June and July), III (August, September and October), and IV (November, December and January).

The HF bulls-sires come from local AI centers, also imported bull semen from European populations of this breed was used. Cows- first calving heifers are kept in tie system and free, depending on the farm. Animals were fed in a usual way depending on the level of intensity of production and the breeding region and the difference was in the quality of feed, i.e. forage to concentrate ratio.

Statistical - mathematical analysis was performed by the Least Squares Mixed Model with fixed and random effects (LS - Least Squares and BLUP - Best Linear Unbiased Prediction) using the LSMLMW software *(Harvey, 1990)*. When analysing the influence of breeding region, year and season of calving on milk performance of dairy cows in first lactation, the following model was used:

$$Y_{ijkl} = \mu + R_l + G_j + S_k + b_1(x_1-x_2) + e_{ijklm}$$

Where:

Yijkl – individual animal (*m*) reared in area (*i*), calved in year (*j*) and season (*k*),
μ = general population average,
OP_l = fixed effect of breeding area (1-4),
G_j = fixed effect of year of calving (1-2),
S_k = fixed effect of the season (1-4),
b_1 = linear regression effect of age at calving,
e_{ijklm} = random error

Results and Discussion

Different number of individual animals in each class, as well as presence of several impacts/factors (breeding region, year of calving, calving season) that influenced the expression of the traits, caused an analysis in which the method of Least Squares was used. Table 1 shows the results of milk traits of cows - first calving heifers. They produced in the first lactation (305 days) 5924 kg of milk with 225.7 kg of milk fat and 190.1 kg of protein

Table 1. The mean (LSM) and errors of mean values (SE) of milk, fat and protein

Trait	QMSL,kg	MFCSL,%	MFYSL,kg	PCSL,%	PYSL,kg
LSM	5923.7	3.81	225.7	3.21	190.1
S.E.	202.7	0.03	7.99	0.02	6.23

Legend: QMSL,kg – quantity of milk in standard lactation, kg (305 days); MFCSL,kg – milk fat content in standard lactation,% (305 days); MFYSL,kg - milk fat yield in standard lactation, kg (305 days); PCSL, kg - milk protein content in standard lactation,% (305 days); PYSL,kg - milk proteint yield in standard lactation,kg (305 days)

Similar data on the Black and White milk cows in first lactation (5658 kg and 3.84% milk fat and 5543 kg and 3.68%) were obtained by *Petrović et al. (1999a)*.

However, Holstein-Friesian heifers, as reported by *Gaidarska et al. (2004)* have realized lower milk yield compared to our results (4300 kg). The superiority of Holstein-Friesian cows in terms of the yield of milk produced is confirmed by results ranging from 6221 kg with 3.57% milk fat to 8500 kg with 3.48% milk fat *(Petrović et al., 1999b; Kuczyńska et al, 2012; Pirlo et al., 2000; De Marchi et al., 2008; Stojić et al., 2011). Pantelić et al. (2011)* have established production of 10245 kg of milk with 3.53% of milk fat, in Holstein-Friesian cows in intensive production of bull dams (Agricultural Corporation Belgrade).

Table 2. The effect of paragenetic factors as LSM deviations ($\hat{c}_i$) of milk traits of cows - first calving heifers

Trait	QMSL,kg	MFCSL,%	MFYSL,kg	PCSL,%	PYSL,kg
μ	5923	3.81	225.7	3.22	190.9
S.E.	202.7	.03	7.99	.02	6.23
Breeding region	$\hat{c}_i$	$\hat{c}_i$	$\hat{c}_i$	$\hat{c}_i$	$\hat{c}_i$
1	334	-0.09	9.9	-0.08	4.9
2	-352	0.07	- 6.9	0.04	-7.8
3	349	-0.08	-11.0	0.08	10.1
4	-503	0.06	- 7.4	0.06	-8.9
5	171	0.03	14.5	-0.09	2.9
ANOVA $F_{exp.}$	**13.563****	**27.256****	**12.498****	**24.563****	**13.471****
Calving year	$\hat{c}_i$	$\hat{c}_i$	$\hat{c}_i$	$\hat{c}_i$	$\hat{c}_i$
1	227	-0.16	-1.2	0.06	9.1
2	-284	0.09	-4.2	0.05	-4.9
3	56	0.07	5.3	-1.1	-3.9
ANOVA $F_{exp.}$	**12.999***	**19.351 ***	**10.658***	**8.856^{NS}**	**9.001***
Calving season	$\hat{c}_i$	$\hat{c}_i$	$\hat{c}_i$	$\hat{c}_i$	$\hat{c}_i$
1	420	- 1.1	5.8	1.6	23.9
2	276	- 0.7	7.9	0.1	11.5
3	-198	0.8	-3.1	-0.9	-14.2
4	-497	0.9	-10.5	-0.8	-21.1
ANOVA $F_{exp.}$	**14.801****	**20.987***	**11.565***	**17.987***	**13.876***

Legend: NS – P>0.05; * – P<0.05; ** – P<0.01; ANOVA - Least – Squares analysis of variance (exp. F)

The sum of a number of factors (paragenetic factors), which constitute a comprehensive term "breeding region" and its effect on milk traits is shown in Table 2. Using the method of Least Squares - fixed model, highly significant deviation was established in yield of milk and milk fat from the average (P<0,01). Thus, cows reared in the region 4 in the first lactation produced 503 kg less milk compared to the general average, and 852 kg less milk than those reared in the third region.

Calving year caused statistically significant deviations in milk yield and milk fat yield (P<0.05), except in case of % protein in milk for which no significant variations (P>0.05) were established. Season in which the cows had calved caused a highly significant deviation in regard to milk yield (P<0.01) and significant deviations in regard to yields of milk fat and protein (P<0,05). Cattle calved in December, January and February have produced 420 kg more milk than the general average, or 917 kg more milk than cows – first calving heifers which calved in the autumn months (Table 2).

Conclusion

Knowledge of the influence of external factors on quantitative traits is very important with respect to their importance in achieving breeding goals and good economic results. Very little research can be found on the results of studying the quantitative milk traits of Black and White Holstein Friesian dairy cattle in Central Serbia, except for a few large herds of Holstein cattle reared in large agricultural companies (near Belgrade). Analysis of the results in this paper suggests that animals of this breed have not achieved satisfactory production according to their genetic potential. The reason for this is inadequate hosuing/rearing, feeding technology and lack of optimal conditions for maximum expression of genetic capacity of the animal. With proper care, nutrition and implementation of zoo-technical measures bred/selected cattle will realize the production in accordance with their genetic potential. This means the genetic improvement of the breed by breeding and selection through quality bulls-sires and systematic improvement of the quantitative genetic traits and continuous work on their improved expression.

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Ispitivanje varijabilnosti osobina mlečnosti populacije holštajn frizijskih goveda u centralnoj Srbiji

M. Lazarević, M.M. Petrović,V. Pantelić, D. Ružić-Muslić, V. Bogdanović, R. Đedović, M.D. Petrović

Rezime

Holštajn goveda se u Evropi gaje uglavnom u nižim predelima. U Republici Srbiji se gaji oko 100.000 krava i junica holštajn frizijske rase Najveći broj gaji se u Vojvodini a manji deo u centralnoj Srbiji. Ako se izuzmu krave koje se gaje u okolini Beograda u vrlo intezivnoj organizovanoj proizvodnji na nekoliko velikih farmi (PKB Padinska Skela, PIK Zemun, BD Agro Dobanovci i dr.), broj koji se gaji na malim farmama u Centralnoj Srbiji je mali i iznosi nekoliko hiljada grla (pod kontrolom mlečnosti manje od 3000). Upravo zbog toga su obavljena naša istraživanja a cilj je bio da ustanovimo kako se u često poluintezivnim uslovima nege i ishrane, ispoljava genetski potencijal kvantitativnih osobina mlečnosti ove rase. U poslednjoj deceniji počelo je gajenje ove rase mlečnih goveda na malim farmama u Centralnoj Srbiji. Tradicionalno, farmeri u ovim regionima su okrenuti proizvodnji mleka i mesa domaće šarene odnosno simentalske rase goveda koja je manje zahtevna u pogledu ishrane, odgoja i koja u manje intezivnim uslovima daje odlične rezultate (mleko, meso).

Podaci obuhvaćeni analizom su prikupljeni u pet regiona (odgajivačka područja) u Cenralnoj Srbiji. Regioni se razlikuju u pogledu geografskih karakteristika a samim tim u proizvodnji hraniva za ishranu krava. Razlike su u načinu i kvalitetu ishrane, menadžmentu u proizvodnji, genetskom kvalitetu grla i dr. Istraživanjem je obuvaćeno 1090 grla krava-prvotelki crno bele odnosno holštajn-frizijske rase. Statističko matematička analiza obavljena je primenom mešovitog modela najmanjih kvadrata sa fiksnim i random uticajima (LS - Least Squares i Blup - Best linear Unbiased Prediction) primenom programa najmanjih kvadrata.

Različit broj individua u pojedinim klasama kao i postojanje više uticaja (region gajenja, godina telenja, sezona telenja) koji su delovali na ispoljavanje ispitivanih osobina , uslovio je analizu u kojoj je korišćen metod najmanjih kvadrata. Prvotelke su u prvoj standardnoj laktaciji (305 dana) proizvele 5924 kg mleka sa 225.7 kg mlečne masti i 190.1 kg proteina. Primenom metoda najmanjih kvadrata, a pomoću fiksnog modela, ustanovljena su visoko značajna odstupanja prinosa mleka, mlečne masti od opšteg proseka ($P<0.01$). Tako su krave gajene u području 4 u prvoj laktaciji proizvele za 503 kg mleka manje u odnosu na opšti prosek i 852 kg mleka manje u odnosu na one koje su gajene u trećem regionu. Godina telenja je prouzrokovala statistički analizirano značajna odstupanja prinosa

mleka i mlečne masti (P<0.05) osim % proteina u mleku gde nisu ustanovljena značajna variranja (P>0.05). Sezona u kojoj su krave bile oteljene prouzrokovala je visoko značajno odstupanje prinosa mleka (P<0.01) i statistički značajna odstupanja prinosa mlečne masti i proteina (P<0.05). Grla oteljena u decembru, januaru i februaru su proizvela 420 kg mleka više u odnosu na opšti prosek ili 917 kg mleka više u odnosu na krave – prvotelke oteljene u jesenjim mesecima.

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EATING QUALITY OF BEEF MEAT

S. Aleksić, M. M. Petrović, V. Pantelić, D. Ostojić-Andrić, N. Stanišić, M. Petričević, D. Nikšić

Institute for Animal Husbandry, 11080 Belgrade, Republic of Serbia
Corresponding author: saleksic@mail.com
Review paper

Abstract: When we talk about the edible quality of beef, the habits of a particular population should first be taken into account. One of the most important traits of beef for consumers is "eating quality". It is difficult to generally define the characteristic "eating quality" because it depends on many factors. These are primarily the factors that affect the traditions and habits of a population of consumers who prepare and consume beef in a certain way. Some consumers prefer meat rich in fat and meat of older animals, while others are quite the opposite which in turn depends on the habits and methods of preparation. The manner of culinary processing is different in a way that particular population of consumers traditionally thermally process beef by cooking, baking, grilling or prefer raw fermented processed meat. The process of globalization has an impact on the harmonization of the criteria for eating quality. World restaurants that are present on the planet, such as McDonald's, Kentucky Fried Chicken, followed by Chinese, Italian, Japanese, Muslim and other restaurants have played major role in this process, especially in the younger population of consumers. The paper presents the most important criteria of beef meat eating quality such as colour, tenderness, softness and flavour of meat. Also analyzed are the most important pre-mortem and postmortem factors that affect the eating quality of meat. These are primarily factors such as the procedure of transportation, "stress syndrome", method of cooling - "cold shortening".

Key words: Beef, beef production, edible quality, meat tenderness, juiciness, flavor, tenderness.

Introduction

One of the most important traits of beef for consumers is "eating quality". It is difficult to generally define the characteristic "eating quality" because it depends on many factors. These are primarily the factors that affect the traditions and habits of a population of consumers who prepare and consume beef in a certain

way. Some consumers prefer meat rich in fat and meat of older animals, while others are quite the opposite which in turn depends on the habits and methods of preparation. The manner of culinary processing is different in a way that particular population of consumers traditionally thermally process beef by cooking, baking, grilling or prefer raw fermented processed meat. The process of globalization has an impact on the harmonization of the criteria for eating quality. World restaurants that are present on the planet, such as McDonald's, Kentucky Fried Chicken, followed by Chinese, Italian, Japanese, Muslim and other restaurants have played major role in this process, especially in the younger population of consumers. The scientific studies have been performed to determine the similarities and differences in beef eating quality of consumers of different countries *(Oliver et al., 2006)*. There are attempts to standardize eating quality using certain instrumental technique. Thus, Quality Meat Scotland made IMEQ project (Integrated Measurement of Eating Quality) where the parameters of pH, temperature, colour, covering of carcass with fat, etc. were integrated. There is also an MSA (Meat Standard Australia) for eating quality that involves parameters such as colour, marbling, fat thickness, age, and pH24.

Production of beef meat

Production of beef depends primarily on the number of cattle. Number of cattle in the world and in Serbia has been steadily declining.

Table 1. Number of cattl in Republic of Serbia

Year	Total number of cattle	Number of cows and pregnant heifers	Category from 3-12 months
1996.	1,325.000	827.919	211.111
2000.	1,246.000	817.000	158.082
2005.	1,079.000	720.000	149.000
2011	937.000	542.000	186.000

Cattle production is an important branch of livestock production in the Republic of Serbia *(Aleksić et al. 2012)*. The data presented in Table 1 shows that over the last 16 years, the number of cattle was continuously declining. This unfavorable situation is compensated by continuously improving the genetic potential of cattle for both milk production and meat production *(Aleksić et al. 2009)*.

Number of slaughtered cattle in the Republic of Serbia in the World

Number of cattle in the Republic of Serbia in the year 2011 totaled 368.000 head of which 38.000 were calves. Total production in the world in year 2010 was 57.323 million tons, of which 12.048.00 tons in USA, Brazil 8.085 million tons, 8.085 million tons in EU, in China 5.600.000 tons. In year 2012, in the People's Republic of China production of beef meat was 5.540.000 tons which is 9.7% of the total world production of beef *(Aleksić et al. 2013).*

Consumption of beef in the Republic of Serbia in the World

The annual consumption of beef in the Republic of Serbia is much lower than the EU average. Consumption of beef per capita in Serbia is on a constant decline, from about 50.2 kg in year 2000 it dropped to 41.4 kg in year 2009. The total consumption of beef in year 2010 in the world was 56 million tons, of which 12.04 million tons in USA, in EU 8.185 million tons, 7.592 million tons in Brazil, and in China 5.589 million tons. As recommended by the World Health Organization (WHO), the amount of meat in the human diet should be 200 g / day or 75 kg / year.

Eating quality of beef meat

When we talk about the edible quality of beef, the habits of a particular population should first be taken into account. One of the most important traits of beef for consumers is "eating quality". It is difficult to generally define the characteristic "eating quality" because it depends on many factors. The Republic of Serbia is a traditional exporter of beef to the Italian market. A typical example of how consumer behavior determines the eating quality is the Italian province of Tuscany. Based on our many years of export practices in this area, Serbia produced beef type "baby beef". This is the meat of female Simmental cattle not older than one year. This market requires the meat that is bright red, juicy and tender. On the other hand, Serbia is exporting beef to the Middle East. Request of this market is primarily to comply with the traditional-religious method of slaughtering *(Aleksić et al. 1999; 2002; 2005)*. Again, in the first place is the most significant factor of edible meat quality is tradition and habit. In general we can say that the edible quality of beef from the point of view of consumers is: colour, tenderness, juiciness and flavour. Meat colour is the first perception that affects the consumer to decide on the edible quality, and only after heat treatment the criteria of eating quality of tenderness, juiciness and flavor of the meat are determined.

Here we will define the basic criteria of eating quality of beef regardless of tradition and habit. These factors can be divided into two basic groups: pre-mortem and post-mortem.

The pre-mortem factors include breed, gender, age, diet, rearing, transport, time spent in the depot immediately before slaughter, etc. Regardless of breed, sex, age, treatment of cattle during transport and at slaughter are very significant for the eating quality of beef *(Alekisić et al. 2006)*. Today this problem is linked to several factors such as the housing of animals especially the pre-slaughter treatment of the animal. The basis of this problem is the stress of cattle, especially their reaction to a new unfamiliar environment during transport and in the slaughterhouse holding pens. Animals exposed to stressors react by secreting hormones by the adrenal glands. The reaction of the body, caused by hormones secreted under stress, is known as the general adaptation syndrome. The immediate consequence of the general adaptation syndrome is the occurrence of DFD meat (dark- firm-dry meat), which are the basic characteristics of the meat. DFD meat is meat of special physical-chemical, technological and other properties whose formation is related to the modern way of rearing and fattening of young cattle for slaughter as well as methods for their commercialization, transport and pre-slaughter procedures. DFD meat is consequence of increased oxygen consumption of the mitochondrial cytochrome oxidase. This condition is permanently stabilized by high pH > 6.0 of meat. Colour of DFD meat is, therefore, color of reduced myoglobin or dark. DFD meat is "firm", which is usually interpreted by its water holding capacity (WHC). Since this is meat from young animals, whose fat content is minimal, which means that a relatively higher content of protein bonds significantly more water, which contributes to a higher strength of meat. However, it is possible that other causes contribute to, or are even the primary factors of firm consistency of DFD meat. One of these reasons is the fact that rigor mortis occurs faster and more complete in DFD meat, and that the resulting actomysine complex is held constant, i.e. the breakdown does not occur as in normal meat. DFD meat, when touched, compared to normal meat is "dry". The reason for this is the low content of free water, because the water, for the most part, is related to the proteins of meat. Also, one of the peculiarities of DFD meat is a high final pH which contributes to the binding of water to proteins, and thus attribute "dry" meat *(Aleksić et al.1995)*. Primarily because of the dark meat such meat is not acceptable to the consumer.

Post-mortem factors such as method of slaughter, cooling, etc., also significantly affect the eating quality of meat. The cooling method can significantly affect a very important feature of eating meat quality – that is tenderness. Fast cooling may contribute to the hardness of meat (the phenomenon of cold shortening). Rapid cooling of carcasses (the phenomenon of cold shortening) occurs when temperature below 15 degrees ND with high pH and high

concentration of ATP. Thus, in temperature conditions of -1 degrees C, cold shortening develops even at pH 7.0. Such meat is of coarse consistency and shows a loss of juice during thermal treatment which is unfavorable from the point of eating quality of meat. There are mechanical processes that contribute to a better eating quality, such as electrical stimulation of carcasses, tumbling of meat, meat injection and thermal treatment.

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Jestivi kvalitet junećeg mesa

S. Aleksić, M.M. Petrović , V. Pantelić, D. Ostojić-Andrić , N. Stanišić, M. Petričević, D. Nikšić

Rezime

Jedna od najvažnijih osobina junećeg mesa za potrošača je jestivi kvalitet - “ eating quality”. Teško je generalno definisati osobinu “eating quality” jer zavisi od mnogih faktora. To su pre svega faktori koji utiču na tradiciju i navike jedne populacije potrošača koji na određen svoj način pripremaju i konzumiraju juneće meso. Neki potrošači preferiraju meso bogato masnim tkivom i meso starijih životinja, dok drugi sasvim suprotno što opet zavisi od navika i načina pripreme. Takođe, način kulinarske obrade se razlikuju tako što određena populacija potrošača tradicionalno juneće meso termički obrađuju kuvanjem, pečenjem, roštiljanjem ili preferiraju sirovo fermentisano obrađeno meso. Proces globalizacije ima uticaja i na ujednačavanje kriterijuma za jestivi kvalitet. Veliku ulogu u tom procesu naročito kod mlađe populacije potrošača Kentucky Fried Chicken, zatim kineski, italijanski, japanski, muslimanski restorani i dr. Vršena su i naučna ispitivanja radi utvrđivanja sličnosti i razlike u jestivom kvalitetu junećeg mesa potrošača različitih zemalja *(Oliver i sar., 2006)*. Postoje pokušaji da se jestivi kvalitet standardizuje određenom instrumentalnom tehnikom. Tako je Quality Meat Scotland napravio projekat IMEQ (Integrated Mesaurment of Eating Quality) gde su integrisani parameter pH, temperatura, boja, prekrivenost trupa masnim tkivom. Takođe postoji i MSA (Meat Standard Australia) za jestivi kvalitet u koji su uključeni parametri boja, marmoriranost, debljina masnog tkiva, starost i pH24 .

Generalno možemo reći da jestivi kvalitet junećeg mesa sa aspekta potrošača su: boja, nežnost, sočnost i aroma. Boja mesa je prva percepcija koja utiče na potrošača da donese odluku o jestivom kvalitetu, tek nakon termičke obrade određuju se kriterijumi jestivog kvaliteta o nežnosti, sočnosti i aromi mesa.

Ovde ćemo definisati osnovne kriterijume jestivog kvaliteta junećeg mesa bez obzira na tradiciju i navike. Ove faktore možemo podeliti u dve osnovne grupe i to: premortalne i postmortalne.

U premortalne faktore spadaju rasa, pol, starost, ishrana, način držanja, transport, vreme provedeno u depou neposredno pre klanja. Bez obzira na rasu, pol, starost tretman junadi u toku transporta i pre klanja su vrlo značajni za jestivi kvalitet junećeg mesa *(Alekisć i sar., 2006)* . Danas se ovaj problem dovodi u vezu sa nizom faktora kao što su način držanja životinja a naročito tretman životinje pre klanja. Osnova navedenog problema je stres junadi , pre svega njihova reakcija na novu nepoznatu okolinu za vreme transporta i boravka u depou klanice. Životinje izložene dejstvu stresora reaguju lučenjem hormona nadbubrežne žlezde. Reakcija organizma, koju izazivaju izlučeni hormoni pod uticajem stresora, označava se kao opšti adaptacioni sindrom. Neposredna posledica opšteg adaptacionog sindroma je pojava DFD-mesa (dark-tamno, firm-čvrsto i dry-suvo), što su i osnovne karakteristike ovog mesa. DFD-meso je meso posebnih fizičko-hemijskih, tehnoloških i drugih svojstava čije je nastajanje povezano sa modernim načinom uzgoja i tova mladih goveda za klanje kao i sa metodama njihove komercijalizacije, transporta i postupaka pre klanja .DFD-meso je posledica povećanog utroška kiseonika od strane citohrom oksidaze mitohondrije. Ovo stanje se trajno stabilizuje visokim pH > 6,0. mesa. Boja DFD mesa je, dakle, boja redukovanog mioglobina odnosno tamna. DFD-meso je"čvrsto" što se obično tumači njegovom sposobnošću vezivanja vode (SVV). Pošto se radi o mesu mladih životinja, čiji je sadžaj masti minimalan, što znači da na relativno veći sadržaj proteina vezuju se i znatno više vode, što doprinosi i većoj čvrstoći mesa. Međutim, moguće je da i drugi razlozi doprinose ili su čak primarni faktori čvrste konzistencije DFD-mesa. Jedan od takvih razloga je i činjenica što *rigor mortis* nastupa brže i potpunije u DFD-mesu i da se tako nastali aktomizinski kompleks održava stalno, tj. ne dolazi do njegovog razlaganja kao u normalnom mesu. DFD-meso je na dodir u poređenju sa normalnim mesom "suho". Razlog za to je mali sadržaj slobodne vode, jer je voda, većim delom vezana za proteine mesa. Takođe, jedna od specifičnosti DFD-mesa je visoki finalni pH što doprinosi boljem vezivanju vode za proteine, a samim tim i atribut "suho" meso. . Pre svega zbog tamne boje mesa ovakvo meso nije prihvatljivo za potrošača.

Postmortalni faktori kao što su način klanja, hlađenje takođe značajno utiču na jestivi kvalitet mesa. Način hlađenja može značajno da utiče na vrlo važnu osobinu jestivog kvaliteta mesa a to je nežnost. Brzo hlađenje može doprineti

tvrdoći mesa (fenomen „cold shortening“). Brzim hlađenjem trupova (fenomen „cold shortening“) javlja se pri temperaturi ispod 15 stepeni i kod visokog pH i visokog sadržaja ATP-a. Tako pri -1 stepen C „cold shortening“ se razvija čak pri pH 7,0. Ovakvo meso je grube konzistencije i pokazuje veliki gubitak soka prilikom termičke obrade što je nepovoljno sa aspekta jestivog kvalieta mesa. Postoje i mehanički postupci koji doprinose boljem jestivom kvalitetu kao što je elektro stimulacija trupova, tamblovanje mesa, injektiranje mesa i termička obrada.

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EXAMINATION OF SULFAMETHOXAZOLE AS ONE OF THE INITIAL SUBSTANCES IN FINISHED MEDICINAL PRODUCT AD US.VET.

V. Vuković[1], J. Kečkeš[1], M. Vićentijević[1], G. Luković[2]

[1]Institute of Veterinary Medicine of Serbia, Auto put 3,11070 Belgrade, Republic of Serbia
[2]Military Medical Academy, Crnotravska 17, 11000 Belgrade, Republic of Serbia
Corresponding author: pjelenna@gmail.com
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Abstract: Pharmaceutical, chemical and biological evaluation of sulfamethoxazole were performed on the basis of legislation. Sulfamethoxazole is the active substance in the finished medicinal product ad us. vet. Aim of this paper is to present the entire process of quality and quantity examination to meet the declared composition when preparing finished product. According to the regulations required testing parameters were: characteristics, identification and content. The reference values for each of the parameters were compared with the results of our examination obtained using prescribed methods and routine tests.

Key words: sulfamethoxazole, quality, a finished medicinal product, regulations, methods

Introduction

Examination of sulfonamides, according to many literature data, is less common than the antibiotics, so we decided to give our contribution to the study of sulfonamides. Also, we have chosen the specific approach - testing sulfonamides as raw material, i.e. starting material for the preparation of the finished product, instead of participating in the finished product as well as clinical trials. From the group of sulfonamides we chose sulfamethoxazole, which is used as a starting substance for preparing finished medicinal products that are used both in veterinary and in human medicine. Sulfamethoxazole in the finished medicinal products, usually with trimethoprim, presents active substance which inhibits normal bacterial utilization of PABA (p – amino benzoic acid) for the synthesis of folic acid, an important metabolite in bacterial resistance to sulfamethoxazole is caused by mutations in the enzymes involved in folic acid synthesis that prevent the drug from binding to it. It is used in treatment of various infections caused by susceptible microorganisms (*Booth and Mc Donald, 1988, Brander, et al., 1991,*

Adams, 1995, Jezdimirović, 1997, Jezdimirović, 2000). Percentage of sulfamethoxazole in the finished products depends on the composition and form of drugs, as well as the purpose of drugs. Daily dosage sulfamethoxazole is up to 25 mg per 1 kg body weight (*Prescott and Baggot, 1994*). The aim of this study was to examine sulfamethoxazole as initial substance, according to the pharmaceutical, chemical and biological requirements (*Law on medicinal products and medical devices, Official Gazette of the Republic of Serbia, no. 30/2010 and 107/2012*). This paper describes the procedure for quality confirmation, based on which further determines if the initial substance is appropriate for the finished product, as well as the required amount of it. Sulfamethoxazole is one of the substances described in the Pharmacopoeia (*Ph. Jug. V, 2000; Ph. Eur., 2006*), which implies that provides a specified test procedure, with appropriate parameters, the prescribed methods and the use of routine tests. Also, there are clearly defined requirements of the specification tests for individual parameters, which are given as a limit values or descriptive. Reference values are the basis for comparing the results obtained in the present study. Only if the prescribed values corresponding to the measured parameters shall be determined quantity of the substance in the finished medicinal product.

Materials and Methods

Control of initial substance is envisaged as a part of documentation for registration of medicinal products (*Ph. Jug. IV, 1984, Ph. Jug. V, 2000*). For that purpose was tested sulfamethoxazole, which is intended for production of the finished product. Prior to examination a detailed study plan was made, including appropriate sample quantity for each tested parameter (*Law on medicinal products and medical devices, Official Gazette of the Republic of Serbia, no. 30/2010 and 107/2012*). As mentioned above in a pharmacopoeia is given the required parameters for sulfamethoxazole (*Ph. Jug. V, 2000; Ph. Eur., 2006*), namely, the parts: Characteristics, Identification, Test and Assay (determination). Furthermore, each of these parameters is explained in detail. So Characteristics include the form, appearance, color and solubility, while the Identification includes Second identification procedure (procedure D). Heavy metals were included within the Test procedure, as well as loss on drying and sulphated ash, while content of sulfamethoxazole was determined under the Assay procedure. Characteristics: form, appearance and color were examined visually. A total of 2 g of the sample was balanced on the watch glass. Sulfamethoxazole must be in the form of crystalline powder, white or almost white. Solubility test procedure was carried out using different solvents. The extent of ***solubility ranges*** from soluble to almost insoluble. In the test tube containing 1 g of sample were added solvents in varying

amounts, depending on the solubility. Distilled water, acetone, alcohol, ether and sodium hydroxide solution were used as solvents. Scale of Evaluation solubility of the sample look like this: soluble - 1 g sample dissolves in less than 2 ml of solvent, freely soluble - 1 g sample is dissolved in 2 up to 10 ml of solvent, sparingly soluble - 1 g sample is dissolved in 10 up to 100 ml of solvent, slightly soluble - 1 g sample dissolves in 100 up to 1.000 ml of solvent, practically insoluble - 1 g sample is dissolved in more than 10.000 ml solvents (*Ph. Jug. IV, 1986; Ph. Jug. V, 2000*). The identification was done by procedure D, by dissolving 5 mg of sulfamethoxazole in 10 ml of 1 M hydrochloric acid. One ml of this solution was transferred into a test tube and diluted with water to 10 ml. Without further acidification with hydrochloric acid, add 0.2 ml of sodium nitrite (100 g/L). After 1 - 2 minutes add 1 ml of β - naphthol, which caused the appearance of a strong orange or red color, and can also form a precipitate of the same color. This reaction gives positive results, i.e. reaction of aromatic amines (*Ph. Jug. V, 2000*). Tests: Determination of lead and cadmium was carried out by atomic absorption spectrometry - AAS (*Analytical Methods for Atomic Absorption Spectrometry, Perkin Elmer instruments*). Loss on drying was determined by drying 1 g of sample in an oven at a temperature of 100 to 105^{o}C in pre-portioned dish (dried under the same conditions). The sample was dried to constant weight, and the maximum allowed is 0.5 % (*Ph .Jug. V, 2000*). Sulphate ash is determined for 1 g of sample. Heat a evaporating dish to redness for 30 minutes, allow to cool in a desiccator and weigh. Place 1 g of sample in the dish, add 2 ml of 2 M sulphuric acid and heat, first on a water bath, then cautiously over a flame and then progressive to 600^{o}C. Continue incineration until all black particles have disappeared and then allow to cool. Add few drops of ammonium chloride solution evaporate to dryness and incinerate carefully. Allow to cool, weigh, incinerate for 15 minutes and repeat this procedure to constant weigh. The maximum allowed is 0.1 % (*Ph.Jug. V, 2000*).

Sulfamethoxazole was determined by using high performance liquid chromatography with UV detection (HPLC - UV). 1 g of sample was weighed and transferred to the flask, 100 ml of methanol was added and the solution was filtered through membrane filter. 20 µl of filtered sample was injected into the system and after the analysis identification of obtained peaks were done on the basis of the retention times of standards of known concentration. Quantification was performed by comparing the peak size (area) of the sample with the peak of a known amount in the standard solution (*Cemal Akay and Sibel A. Özkan, 2002*). After all of examination the obtained results were compared with reference values, after which has been undertaken assessment of sulphamethoxasole as the starting material.

Results and Discussion

All determination is done using prescribed methods, and the results were evaluated in comparison with reference values. Table 1 shows the obtained results along with reference values.

Table 1. Obtained results

	Parameter	Reference value	Result
1	Characteristics		
1.1.	Form	powder	corresponds
1.2.	Appearance	crystalline	corresponds
1.3	Color	white or almost white	white
1.4.	Solubility	Solubile in sodium hydroxide solution	solubile
		freely solubile in acetone	freely solubile
		sparingly solubile ina alcohol	sparingly solubile
		slightly solubile in ether	slightly solubile
		practically insolubile in water	practically insolubile
2.	Identification	gives the reaction of primary aromatic amines	corresponds
3.	Test		
3.1.	Heavy metals	< 20 mg/kg	< 0,5 mg/kg
3.2.	Loss of drying	up to 0,5 %	0,48 %
3.3.	Sulphated ash	up to 0,1 %	0,09 %
4.	Assay	99,0 - 101,0 %	100,89 %

Examination of characteristics, as one of the parameters was carried out in an appropriate way, including all the require specifications for the starting substance. The table 1 shows that the required form is powder which corresponds to the obtained result. Also, visually was confirmed crystalline appearance and the color was white (Table 1). Solubility testing included a total of 5 different solvents. For dissolving 1 g of sulfamethoxazole, less than 2 ml of NaOH solution were spent, while the sample was easily soluble in acetone. Alcohol has proven to be a weak solvent for sulfamethoxazole, which was slightly soluble in ether, and practically insoluble in water (Table 1). Identification procedure was D, the so-called second identification. The procedure is based on the reaction of primary aromatic amines, which confirmed that the tested sample corresponds to the requirements. Two heavy metals were tested: lead and cadmium. Examination procedure was carried out using AAS, and the obtained value of less than 0.5 mg/kg is within the requirements for heavy and toxic metals. The result for the loss of drying was 0.48 %, and 0.09 % for sulphated ash, which corresponds to the

prescribed values. Determination of sulfamethoxazole was performed using high performance liquid chromatography and the obtained value was 100.89 %, which was within the prescribed limit values (Table 1).

Conclusion

From the performed examination the following can be concluded:

- All examined parameters were in accordance to the requirements (*Ph. Jug. V, 2000; Ph. Eur., 2006)*
- Sulfamethoxazole is an appropriate starting material for the preparation of the finished product
- In adequate storage conditions, on the basis of the assay, it is easy to assemble the finished medicinal product according to the amount prescribed in the declared composition.

Ispitivanje sulfametoksazola kao jedne od polaznih supstanci za dobijanje leka ad us.vet.

V.Vuković, J. Kečkeš, M.Vićentijević, G.Luković

Rezime

U okviru farmaceutsko-hemijsko-biološke dokumentacije o kvalitetu, ispitan je sulfametoksazol, kao polazna supstanca koja predstavlja aktivni princip u gotovom leku ad us.vet. Osnovni cilj ispitivanja je da se prikaže celokupna procedura ispitivanja, provera kvaliteta i kvantiteta za zadovoljenje deklarisanog sastava pri spravljanju gotovog leka. Prema propisima zahtevani parametri su: Osobine, Identifikacija i Određivanje. Pri tome, za svaki parametar su date referentne vrednosti sa kojima su upoređivani rezultati naših ispitivanja do kojih se došlo korišćenjem propisanih metoda i rutinskih testova.

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PRESENCE AND SOURCES OF FAECAL CONTAMINATION OF DRINKING WATER SOURCE

S. Ćirić[1], Z. Ilić[1], B. Milošević[1], J. Stojković[1], Z. Spasić[1], N. Stolić[2], B. Ristanović[1]

[1] Faculty of Agriculture, Lešak, Republic of Serbia
[2] College of Agriculture and Food Technology, Prokuplje, Republic of Serbia
Corresponding author: slavica.ciric@open.telekom.rs
Original scientific paper

Abstract: The main sources of faecal contamination of drinking water sources are animal and human waste. In order to determine appropriate measures for protection and remediation of drinking water source, it is necessary to know from where the pollution originates. For this purpose, sampling of the raw water (surface water) that is processed into drinking water was carried out. The following parameters of water were examined: temperature, turbidity, oxygen concentration, the concentration of inorganic nitrogen, total count of heterotrophic bacteria, number of faecal coliforms (FC) and number of faecal streptococci (FS). As an indicator of faecal contamination origin, the FC/FS ratio was determined. The value of this ratio ranged from 1.10 in February to 5.81 in October. The dominance of human waste was recorded in 40.4% of samples, while the dominance of animal waste (domestic animals) was noted in 36.2% of samples. In the summer the livestock waste was dominated, and in the fall the water was polluted with human waste mainly.

Key words: drinking water, faecal contamination, FC/FS ratio, human waste, animal waste

Introduction

Faecal contamination in water occurs from three main sources, livestock, wildlife and humans. Besides being potential pathogens, faecal bacteria can indicate the presence of human viruses, while bacteria from wild and domestic animals may indicate the presence of protozoal parasites and the presence of the species of genus *Campylobacter* that cause diseases in humans and animals *(Ivanović, 2008)*. Knowing the sources of bacteria in water body or water supply is of great value in the remediation and prevention of further bacterial contamination

(http://www.epa.gov/). Agriculture is listed as the primary source of contamination *(Graves, 2003)*.

The single most important indicator of public health hazard from infectious agents is the occurence of faecal coliform bacteria in water. Faecal streptococci have received widespread acceptance as useful indicators of microbiological water quality, because: they show a high relationship with health hazards, they are always present in feces of warm-blooded animals and their die off is less rapid than that of coliforms in water *(Richardson et al., 1991)*.

The ratio of faecal coliform (FC) to faecal streptococci (FS) count has been used to differentiate human from non-human sources of faecal contamination *(Coyne and Howell, 1994; Edwards et al., 1997)*. This method is an inexpensive, moderately complicated laboratory procedure *(Sargeant, 1999)*. In human wastes, the FC/FS ratio is greater than 4.0. In mixed pollution with human waste domination, the ratio is between 2.0 and 4.0. In mixed pollution with animal waste domination (domestic animals), the ratio is between 0.7 and 2.0. If the domestic animals are the only sources of faecal contamination, the ratio is less than 0.7 *(Gerba, 1999)*.

The aim of this study was to investigate the presence, seasonal variability and origin of faecal contamination of water that is used as drinking water after treatment. Also, the goal was to determine the significance of differences in values of major physico-chemical and bacteriological parameters of water between seasons, as well as to examine the correlation between the FC/FS ratio and other parameters.

Materials and Methods

During the one-year investigation the sampling of native raw water at the Kruševac waterworks catchment site was conducted. It was done once a week. Native raw water is water that is taken from the hypolimnion (about 20 m depth) from catchment basin of Ćelije Reservoir (central Serbia). This water is sent to the Drinking Water Treatment Plant and from there distributed to consumers of the town of Kruševac and its surroundings.

On the site, the water temperature was recorded using a mercury thermometer with 0.2 °C precision. Physico-chemical parameters: turbidity (NTU), the concentration of O_2 (mg/l) and the concentration of inorganic nitrogen (mg/l), were determined by American standard methods *(APHA-AWWA-WEF, 1998)*.

The total count of heterotrophic bacteria was determined by the cultivation method with inoculation of the samples on R2A medium *(Merck, 2005)*. Grown colonies were counted after incubation at 20 °C for 72 hours. Each sample was inoculated in three dilutions: 10^{-1}, 10^{-2} and 10^{-3}. Enumeration of bacteria was

performed on plates inoculated with lowest dilution that could be read. The obtained number is converted to 1 ml of sample and expressed as colony forming units per mililiter (cfu/ml). The number of faecal coliforms and faecal streptococci were determined by standard methods *(APHA-AWWA-WEF, 1998)*. On the basis of these values, the FC/FS ratio was calculated.

Using the t-test, the significance of differences in the values of basic physico-chemical and bacteriological parameters between seasons was examined. By the correlation analysis the existance and degree of correlation between the FC/FS ratio and other measured parameters were tested. Both of these statistical methods were performed using software STATISTICA v.8.0, StatSoft, Inc.

Results and Discussion

Results of the basic physico-chemical and bacteriological analysis of raw water are shown in Table 1. All parameters, except temperature, reached maximum values in spring (March and April). The temperature was highest in summer and autumn. The lowest values of the investigated parameters were recorded in October, except temperature which was the lowest in March, and the number of heterotrophs, which was the lowest in August.

Table 1. Results of physico-chemical and bacteriological analysis of water

Parameter	Month												Σ
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	
Temperature (°C)	4.9	4.4	3.9	9.1	11.8	13.5	14.8	14.8	14.1	15.3	11.1	9.2	10.9
Turbidity (NTU)	1.63	2.18	16.50	27.50	5.68	12.55	9.66	4.83	3.36	1.58	2.30	3.03	7.57
O_2 (mg/l)	5.03	11.13	11.66	11.07	9.35	5.71	4.15	1.72	1.39	0.88	4.04	5.08	5.88
N-mineral (mg/l)	1.170	1.244	1.817	1.473	1.532	1.376	1.220	1.197	1.037	0.586	0.596	0.765	1.164
HPC (cfu/ml)x10^3	1.32	9.00	23.43	21.04	2.81	10.58	1.86	0.73	5.83	2.16	1.14	1.58	6.79

HPC-Heterotrophic plate count; cfu-colony forming units

Indicators of faecal contamination of water were constantly present in the water which is a proof of constant and long-term pollution. Numbers of faecal coliforms and faecal streptococci by month are shown in Figure 1. The number of faecal coliforms ranged from a minimum of 2/100 ml in August and September, to a maximum of 215/100 ml in April. In the same months, the minimum (1/100 ml) and maximum (59/100 ml) values of faecal streptococci were recorded. The highest faecal contamination of water was observed in the spring (March and April), when

the maximum values of turbidity, inorganic nitrogen and total heterotrophic bacteria were also registered (Table 1).

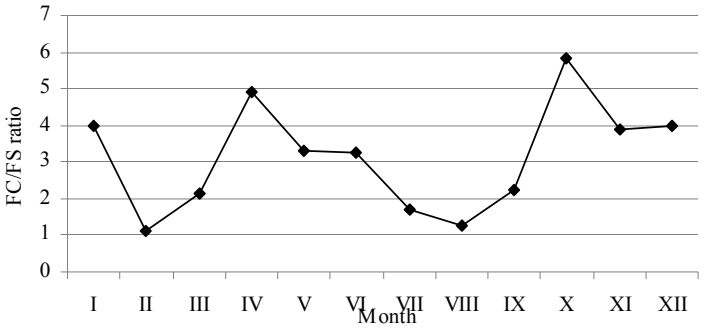

Figure 1. Numbers of faecal coliforms and faecal streptococci in water

The significance of differences in values of basic physico-chemical and bacteriological parameter between seasons is shown in Table 2. Faecal coliforms were significantly more abundant in the spring than in summer and autumn. In the same seasons significant differences in abundance were shown by heterotrophs and faecal streptococci.

Table 2. Results of t-test for some physico-chemical and bacteriological parameters of water between seasons

		Temp. (°C)	Turbidity (NTU)	HPC (cfu/ml)	FC (cfu/100ml)	FS (cfu/100ml)	FC/FS
s vs. su	t	5.48*	1.56	2.28*	2.08*	2.26*	1.97
	p	0.000	0.133	0.032	0.049	0.033	0.061
s vs. a	t	4.50*	3.24*	2.87*	2.88*	3.02*	0.67
	p	0.000	0.000	0.009	0.008	0.006	0.510
s vs. w	t	2.89*	2.42*	1.41	2.02	2.15*	1.09
	p	0.009	0.026	0.175	0.058	0.046	0.290
su vs. a	t	1.07	5.50*	0.49	1.19	1.63	2.19*
	p	0.300	0.000	0.627	0.248	0.117	0.038
su vs. w	t	16.36*	4.28*	0.33	0.73	1.06	0.36
	p	0.000	0.000	0.742	0.476	0.306	0.720
a vs. w	t	11.33*	0.63	0.79	0.38	1.63	1.34
	p	0.000	0.534	0.441	0.712	0.117	0.200

FC-faecal coliforms, FS-faecal streptococci, s-spring, su-summer, a-autumn, w-winter
* the difference is significant (95% confidence)

On the basis of the representation of faecal coliforms, 54% of water samples fall in slightly polluted, 30% of samples in less polluted waters, and the remaining of 16% of samples in moderately polluted waters *(Kavka, 1994)*.

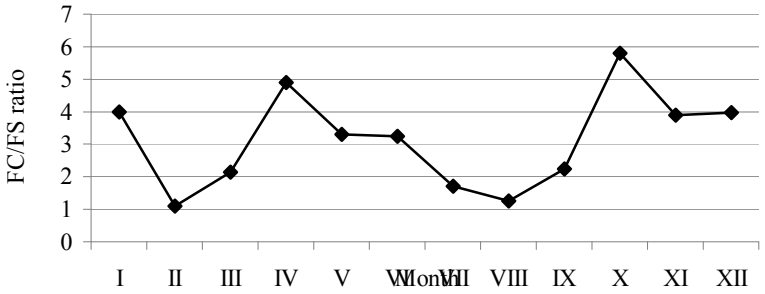

Figure 2. FC/FS ratio in water

As an indicator of the origin of faecal contamination of water the ratio of the numbers of faecal coliforms and faecal streptococci is recommended *(Coyne and Howell, 1994; Edwards et al., 1997)*. The values of this ratio are shown in Figure 2. It ranged from 1.10 in February to 5.81 in October.

Faecal coliforms and faecal streptococci were present along in 94% of water samples (Figure 3). Ratio of FC/FS greater than 4.0, indicating the presence of human waste only, was noted in 23.4% of samples (Figure 4), while the ratio less than 0.7, indicating the presence of animal waste only, was not recorded in any sample. Ratio of FC/FS greater than 2.0 and less than 4.0, indicating a mixed pollution with domination of human waste, was registered in 40.4% of samples. Ratio greater than 0.7 and less than 2.0, showing a mixed pollution with domination of animal waste (domestic animals) was noted in 36.2% of samples (Figure 4). These results show that the source of faecal contamination have never been exclusively animals, but that the most common cause of this type of pollution (in 76.6% of samples) was a combination of human and animal waste. Similar results have been obtained by other authors *(Zmyslowska and Golaś, 2003)*.

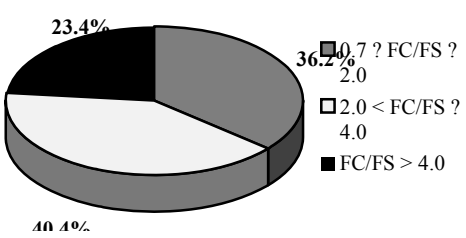

Figure 3. Percentages of water samples on

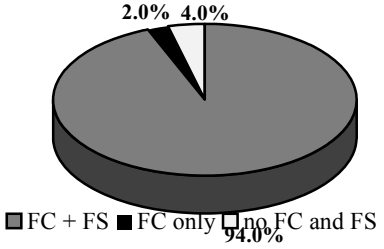

Figure 4. Percentages of water samples based on

the basis of the presence of FC and FS FC/FS ratio

Significant difference in FC/FS ratio was recorded between summer and autumn (Table 2), while in the summer the livestock waste was dominated, and in autumn the water was polluted with human waste mainly.

Results of correlation analysis of the relationship between FC/FS ratio and some physico-chemical and bacteriological parameters of water are given in Table 3. The only significant correlation of this ratio was registered with the concentration of mineral nitrogen, ie. with the increase of concentration of nitrogen the value of FC/FS ratio was also increasing.

Table 3. Results of correlation analysis between FC/FS ratio and some physico-chemical and bacteriological parameters of water

vs.	Temperature (°C)	Turbidity (NTU)	O_2 (mg/l)	N-min. (mg/l)	HPC (cfu/ml)
FC/FS	r = 0.11 p = 0.429	r = 0.04 p = 0.765	r = 0.13 p = 0.374	r = 0.29* p = 0.038	r = 0.01 p = 0.964

* significant correlation between parameters exists

Conclusion

The results of this study show constant and long-term faecal contamination of the tested drinking water source. Abundance of faecal coliforms and faecal streptococci reached maximum in April, 215/100 ml and 59/100 ml, respectively, and minimum in August and September, 2/100 ml and 1/100 ml, respectively. Indicators of faecal contamination were significantly more abundant in the spring than in summer and autumn.

Ratio of FC/FS ranged from 1.10 in February to 5.81 in October. Source of faecal pollution of water have never been exclusively animals, but the most common cause of pollution was a combination of animal and human waste (in 76.6% of samples). Waste of domestic animals dominated in the summer, while in autumn the water was polluted with human waste mainly.

Based on these results, a plan of protection of drinking water source can be created.

Acknowledgement

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Prisustvo i poreklo fekalne kontaminacije izvorišta vode za piće

S. Ćirić, Z. Ilić, B. Milošević, J. Stojković, Z. Spasić, N. Stolić, B. Ristanović

Rezime

Osnovni izvori fekalne kontaminacije izvorišta vode za piće su humani i animalni otpad. Da bi se odredile adekvatne mere zaštite i sanacije izvorišta neophodno je znati poreklo zagađenja. U tom cilju izvršeno je uzorkovanje sirove vode koja se prerađuje u vodu za piće. Ispitani su sledeći fizičko-hemijski i bakteriološki parametri vode: temperatura, mutnoća, koncentracija O_2, koncentracija mineralnog azota, ukupan broj heterotrofnih bakterija, broj fekalnih koliforma (FC) i broj fekalnih streptokoka (FS). Kao pokazatelj porekla fekalne kontaminacije određen je odnos FC/FS. Vrednost ovog odnosa se kretala od 1,10 u februaru do 5,81 u oktobru. Dominacija humanog otpada beleži se u 40,4% uzoraka, dok se dominacija animalnog otpada (domaće životinje) beleži u 36,2% uzoraka. U leto dominira otpad domaćih životinja, dok je u jesen voda zagađena pretežno humanim otpadom.

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MICROBIOLOGICAL AND EPIZOOTOLOGICAL CHARACTERIZATION OF MATERIALS FROM WASTEWATER TREATMENT PLANT

T. P. Popova[1], M. D. Kaleva[1], B. D. Baykov[2], B. S. Zaharinov[2], S. M. Marinova-Garvanska[3]

[1]Faculty of Veterinary Medicine, University of Forestry, Kliment Ohridski Blvd. 10,Sofia1756, Bulgaria
[2]New Bulgarian University, Montevideo Blvd. 21,Sofia 1618, Bulgaria
[3]Institute of Soil Science, Agricultural Technologies and Plant Protection "N. Pushkarov" Shosse Bankia Street 7, Sofia1080, Bulgaria
Corresponding author: dr_tpopova@abv.bg
Original scientific paper

Abstract: Studies were carried out for the presence of microorganisms of major groups, containing pathogenic agents with epizootological importance, in order characteristic of their dynamics at different levels in the treatment plant and assessing the environmental safety of the final materials. For this purpose were traced the changes in the quantities of microorganisms contained in the materials from successive levels of processing. It was found that the purified water inlet and outlet does not contain *Salmonella enterica*. Presence of *Escherichia coli* and *Clostridium perfringens* beyond the requirements of the regulation *(Decree N339, 2004)* was not established, as well as *Enterococcus* spp. The tested sludge contained microorganisms from these groups and could not be deposited in the soil without prior aerobic or anaerobic processing. The draining in the nature of treated water at the outlet of plant does not hide environmental risk. In addition to monitoring of the sanitary indicative bacteria, the following of the main groups of Gram-negative and Gram-positive microorganisms in the water and sewage sludge allows more reliable assessment of their decontamination. Furthermore, the new approach for reporting the results in a unit of dry matter of the studied materials, proposed here, allows their more exact comparison.

Key words: waste water treatment plant, microflora, epizootological assessment

Introduction

The wastewater treatment is a contemporary method for their manufacture. Its application is important for protection of the environment (soil and water) from contamination by toxic substances, heavy metals and causes of infections that may be contained in such water. This is essential for the support healthy and stable ecosystems. The main function of the plant is to accelerate the natural process of self-purification of water. Due to the growth of population, industry and livestock, such treatment is required for accelerating the purification of the large amounts of wastewater *(EPA, 2004)*.In order to prevent the spread of pathogens is important their reduction or inactivation in the final products, postponed in nature. The most common possibilities for recast include anaerobic degradation, aerobic digestion and composting. Incineration also is used, but to a much lesser extent *(Langenkamp and Part, 2001; EPA, 2004)*.

The aim of this work was to perform a microbiological assessment of the wastewater and sludge of individual purification steps in urban wastewater treatment plant in terms of their epizootiological safety.

Materials and Methods

Samples from different stages of processing in urban wastewater treatment plant near to Sofia, obtained during the first half of April, were examined. The materials were indicated as follow: incoming water after precipitating (IW); primary sludge (PS); secondary sludge (SS); mixed sludge (MS), dewatered by belt filter presses; stayed mixed sludge (SMS); input into the digester (methane tank) (ID); purified water (PW) in the output of the plant.

Microbiological studies were conducted in accordance with the Ordinance on the terms and conditions for use of sludge from wastewater treatment through its use in agriculture *(Decree N339, 2004)*. The titers of *Escherichia coli* and *Clostridium perfringens* also were established. Additionally were tracked the quantities of bacteria from the genera *Staphylococcus, Enterococcus, Pseudomonas*, Gram-negative aerobic bacteria, fungi, and the total number of microorganisms.

Selective media (Scharlau - Antisel, Bulgaria) were used for isolation and quantitative determination of the microorganisms from the studied groups and types. The following media have been chosen: Mueller Hinton agar for counting the total number of microorganisms in the examined material, Eosin Methylene Blue agar for *E. coli* and Gram-negative aerobic bacteria, Cetrimide agar for bacteria of the genus *Pseudomonas*, Chapman Stone agar for those of the genus

Staphylococcus, Sabouraud agar for fungi, selective medium for enterococci, Salmonella-Shigella agar for *Salmonella enterica* and selective agar for *Clostridium perfringens* (Merck -Bio Lab, Bulgaria).

Quantitative determination of the microorganisms was carried out using the conventional method in serial tenfold growing dilutions of the investigated material in a sterile saline solution. Cultures of them were made on the selected media, three for each medium and dilution. After incubation at 37° C for 24-72 h under aerobic and anaerobic conditions (with Anaerocult ® A mini - Merck-Bio Lab, Bulgaria) the mean arithmetical number of developed colonies was determined and the quantities of colonies forming units (CFU-colony forming units) in 1 ml or 1 g of starting material were calculated. The corresponding quantities of microorganisms in 1 g dry matter in each of the studied materials also were calculated. For this purpose, the number of detected CFU was multiplied by the quotient obtained according to the percentage of dry matter in the material.

Results and Discussion

The results of the studies of the quantitative changes in the total number of microorganisms, as well as of Gram-negative bacteria in the materials from the various stages of treatment plant, are presented in Figure 1.

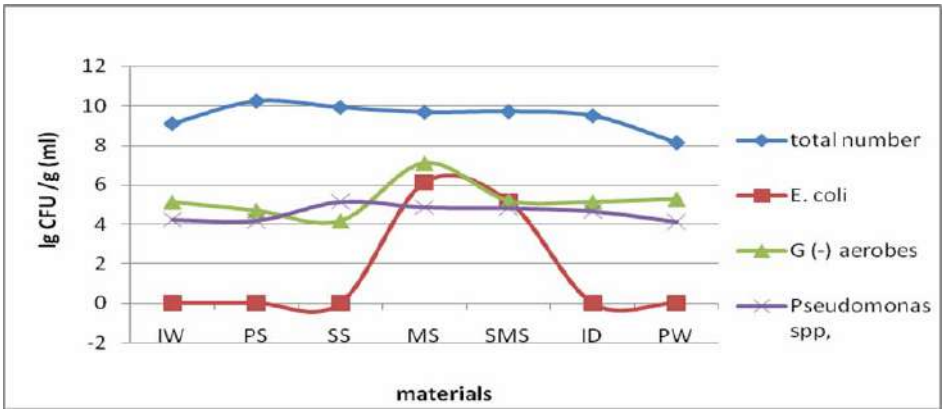

Figure 1. Quantities of microorganisms (total and Gram-negative bacteria) in the examined materials of the various steps of the wastewater treatment plant. IW - incoming water after precipitation; PS - primary sludge; SS - secondary sludge; MS - mixed sludge, dewatered; SMS - stayed mixed sludge; ID - input into the digester; PW - purified water in the output of the plant.

The graphs of Figure 2 show the quantitative change of the microorganisms from the same groups, represented in a unit of dry matter in the materials.

The content of dry matter in the tested samples in % was as follows: IW - 0,00049; PS – 4,9; SS – 0,9; MS – 41,81; SMS – 56,46; ID – 2,96; PW – 0,00048.

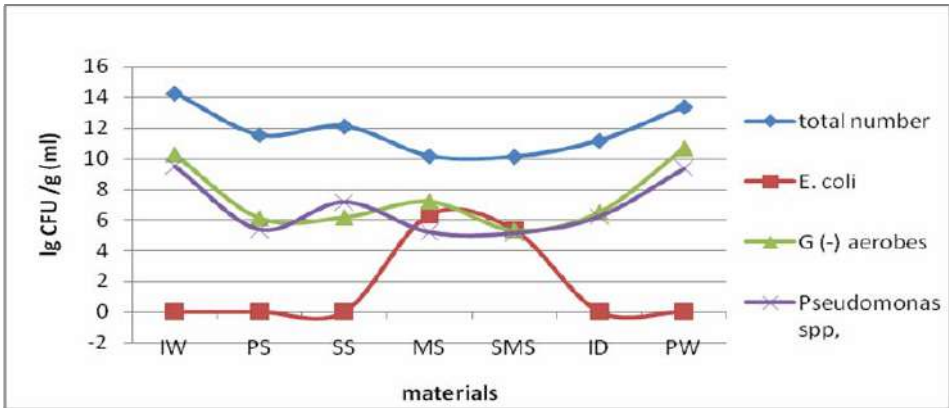

Figure 2. Quantities of microorganisms (total and Gram-negative bacteria), presented in a unit of dry matter in the materials of the wastewater treatment plant. IW - incoming water after precipitation; PS - primary sludge; SS - secondary sludge; MS - mixed sludge, dewatered; SMS - stayed mixed sludge; ID - input into the digester; PW - purified water in the output of the plant.

Figures 3and 4 show the data from the quantitative studies of Gram-positive organisms in the samples from the steps of the treatment plant, presented in a unit fresh and dry material respectively.

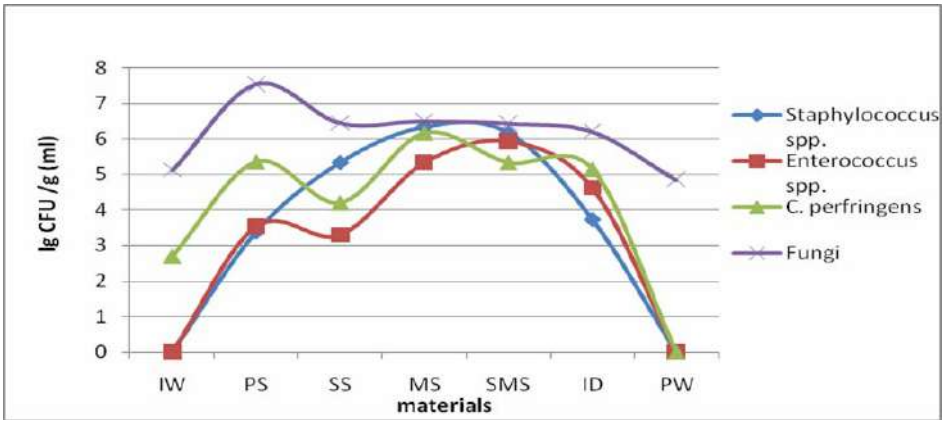

Figure 3. Quantities of Gram-positive microorganisms in the examined materials from various stages of the wastewater treatment plant. IW - incoming water after precipitation; PS - primary sludge; SS - secondary sludge; MS - mixed sludge, dewatered; SMS - stayed mixed sludge; ID - input into the digester; PW - purified water in the output of the plant.

From the graphs of the figures is seen that the number of saprophytic microorganisms was higher in the materials with high water content. Sanitary indicative bacteria showed relatively high levels in the examined sludge.

Salmonella enterica was not isolated from the studied materials at all levels of recast. In the examined water samples (input and output) *E. coli* and *Clostridium perfringens* were not established in quantities exceeding the set out in the requirements of Bulgarian current Ordinance on the manner of utilization of sludge from wastewater treatment through its use in agriculture *(Decree N339, 2004)*. From these samples was not isolated and *Enterococcus* spp. Draining in the nature of the treated water at the output of plant does not hide environmental risk. As shown in the data of other authors *(Langenkamp and Part, 2001; Talahassee, 2010)*, subject to the requirements of modern techniques for purifying of wastewater, the risk of waterborne diseases greatly reduced.

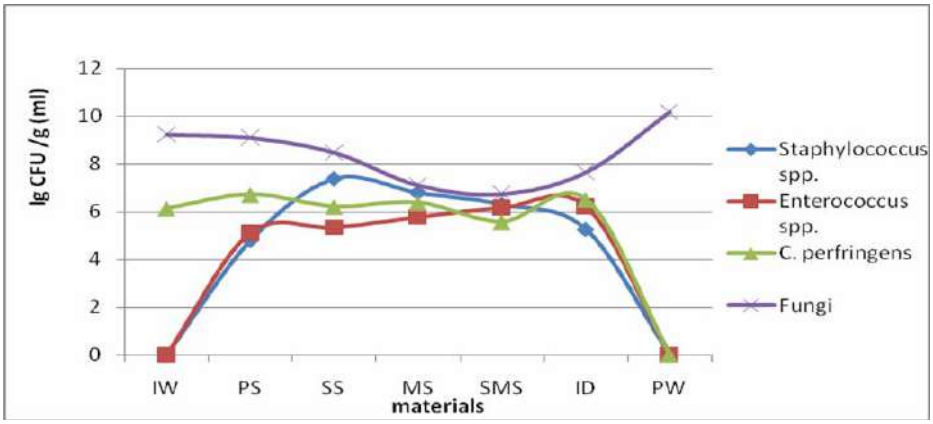

Figure 4. Quantities of Gram-positive organisms, presented in a unit of dry matter of the examined materials. IW - incoming water after precipitation; PS - primary sludge; SS - secondary sludge; MS - mixed sludge, dewatered; SMS - stayed mixed sludge; ID - input into the digester; PW - purified water in the output of the plant.

The studied sludge, however, contained relatively high amounts of microorganisms from these groups (*E. coli, Clostridium perfringens, Enterococcus* spp.) and should not be deposited in the soil without prior aerobic or anaerobic processing. As can be seen from the data presented, the quantities of microorganisms from all monitored groups increased in primary and secondary sludge, as their content was highest in the mixed sludge. The established high microbial content in the activated sludge is natural, since it is related with the role of these microorganisms in the aerobic decomposition of the organic compounds, which is carried out at this stage of purification. The incoming into the digester material contained microorganisms from the groups with pathogenic representatives in amounts less with about 1 lg compared to these in previous level of processing. During anaerobic digestion their quantity is subjected to further reduction on account of the development of specific anaerobic microbial types. The dynamics of the fungi was closely related to that of the total number of microorganisms. Similar were the graphs of motion of Gram-negative aerobes, staphylococci, enterococci and *Clostridium perfringens*.

In our view, the representation of the results in a unit of dry matter of the studied materials, proposed here by us, allows their more exact comparison.

These results demonstrate that the practice of landfilling the final dewatered sludge is necessary and proper from an environmental perspective as its microbial content is high, including sanitary illustrative families and kinds of bacteria. The incoming in the bioreactor material was with a lower content of microorganisms from all traced groups, compared to the previous levels. Our former studies *(Popova et al., 2009)*, associated with the decontamination of

organic material from livestock under continuous mesophilic anaerobic degradation process, showed that the resulting compost was safe from the epidemiological point of view and could be used to fertilize the soil. Fastest and most reliable results in this direction gives the thermophilic anaerobic degradation mode, as well as mesophilic regime, combined with pasteurization of input and output material *(Popova et al., 2012)*.

Conclusion

Tracking of the main groups of Gram-negative and Gram-positive microorganisms in the wastewater and sewage sludge enables more reliable assessment of their decontamination.

The proposed new approach for reporting of the results in a unit of dry matter of the tested materials allows a more accurate comparison of the content of microorganisms therein.

At abidance of technology of purification, the treated water at the exit of the plant does not contain *E. coli*, enterococci and *Clostridium perfringens* and its discharge into natural waters is safe.

The studied sludge contained microorganisms from these species and can't be deposited in the soil without prior aerobic or anaerobic processing.

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Mikrobiološka i epiziootološka karakterizacija materijala iz postrojenja za prečišćavanje otpadnih voda

T. P. Popova, M. D. Kaleva, B. D. Baykov, B. S. Zaharinov, S. M. Marinova-Garvanska

Rezime

Istraživanja su sprovedena na prisustvo mikroorganizama velikih grupa, koje sadrži patogene agente epizootološkog značaja, u cilju utvrđivanja

karakteristika njihove dinamike na različitim nivoima u postrojenju i procene ekološke bezbednosti završnih materijala. U tu svrhu su se pratile promene u količini mikroorganizama koje se nalaze u materijalima sa sukcesivnim nivoima obrade.

Utvrđeno je da prečišćeni ulaz i odvod vode ne sadrži *Salmonela enterica*. Prisustvo *Escherichia coli* i *Clostridium perfringens* izvan zahteva propisa (Uredba N339, 2004) nije osnovana, kao i *Enterococcus* spp. Testiran mulj sadrži mikroorganizme iz ovih grupa i ne može da se deponuje u zemljištu bez prethodnog aerobnog ili anaerobnog tretmana. Odvođenje u prirodu tretirane vode na izlazu iz postrojenja ne krije rizik po životnu sredinu.

Pored praćenja sanitarno indikativnih bakterija, praćenje glavne grupe gram-negativnih i gram-pozitivnih mikroorganizama u vodi i kanalizacionom mulju omogućava pouzdaniju procenu njihove dekontaminacije. Osim toga, novi pristup za izveštavanje rezultata po jedinici suve materije ispitivanih materijala, predloženo u radu, omogućava njihovo preciznije poređenje.

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AGROECOLOGIC VALUATION OF ORGANIC WASTE IN DIFFERENT TECHNOLGIES OF STORAGE

B. Zaharinov[1] Z. Shindarska[2], S.Marinova - Garvanska[3], V. Kirov[2], B.Baykov [1]

[1] New Bulgarian University, h.c. " Ovcha Kupel", St. Montevideo 21, 1618 Sofia,Bulgaria
[2]University of Forestry, 10, St.Kl.Ochridsky Blvd., 1756 Sofia, Bulgaria
[3]Institute of soil Science " Nikola Pushkarov, St. Shosse "Bankya", 1080 Sofia, Bulgaria
Corresponding author: zshindarska@hotmail.com
Original scientific paper

Abstract: A study has been conducted, based on three types of fertilizers / cow, pig and bird manure/ and bioshlam /received from clearing station/ that have been storage in different technologies in order to make an agroecologic valuation. The different types of fertilizers and wastewater in different technologies of storage have been analyzed according to the following parameters: Dry matter (DM) ,Organic matter (OM), Organic Carbon (OC), Nitrogen Kjeldahl (N), Nitrogen Ammonium (N -ammonium), Nitrogen nitrate (N- nitrate), Sulfates (SO_4) , Calcium Oxide (CaO), Magnesium Oxide (MgO), Patassium (K), P (Phosphorus), pH (H_2O) and heavy metals – arsenic (As), Cadmium (Cd), Chromium (Cr), Copper (Cu), Mercury (Hg), Nickel (Ni), Lead (Pd), Zinc (Zn). The received results show: variation of the percentage of dry material /from 92,12% to 4,03%/ according to the technology of storage. The way of storage has no essential influence on the content of carbon in the various stable fertilizers, where pig manure has the highest value of nitrogen in all technologies of storage. Near to the recommendable optimal values of C:N is stayed/dry/ and fresh/hard/ in all three fertilizer types. The technology of storage by the different manure types has no influence on the content of heavy metal. Exception can be observed as far as the zinc content in stale /fluid/ pig manure is concerned. High content of dry material can be observed in bioshlam received from clearing station. In both ways of production of variation in the content of dry material the ratio between the biogenic macro elements C:N remains. The free nitrogen forms (N-ammonium and N-nitrate) in bioshlam are higher to other organic waste in both technologies of storage. No values over the critically admissible in bioshlam are observed in both technologies of production.

Key words: cow manure (fertilizer), Pig manure, Bird manure, Technology for storage, agri- environmental indicators (assessment),waste water treatment plan, bioshlam

Introduction

In recent years a growing interest in organic waste received by livestock animals, grew due for environmental and agrochemical problems while they technological applications of anaerobic digestion as a raw material for biofuel production.

The energy content of manure (regardless of what king of animals received is approximately 50%) that of the feed. Manure containing a high concentration of ammonia which has an inhibitory effect on the glycolitic pathway (*Baykov, et al., 2007*).With simultaneous decomposition of plant materials and fertilizers. Manure provides buffering capacity and a wide range of food substances, till added plant material high carbon improves the balance of the elements C: N (*Lehtomaki et al., 2007*). Differences in the ratio of C: N allow combine fertilizers with regard to the need of nitrogen in the soil, as well as the requirements of the EU directive Nitrate (1991) to increase the soil fertility by limiting the amount of nitrogen contributed to 170 kg /ha year EU Directive and regulation 22/2010.

The present work aims to make agri-environmental assessment of fertilizers from various livestock animals and bioshlam of Treatment plant (TP) at different storage technologies to shape substrates with their participation with good agri-environmental indicators.

Materials and Methods

The study included:

1.Collecting of samples of different fertilizers - beef, pork and chicken and compost from (Sofia) at various storage technologies. The Table 1 shows the types of fertilizers and compost (sludge) at various storage technologies.

2. Analysis of samples for the following parameters and methods: Dry matter (DM) by BSS EN 14346 (Bulgarian State Standart), Organic matter (OM) determined based on estimates of BOD, Organic Carbon (OC) by BSS EN 13137, Nitrogen Kjeldahl (N) by BSS 13342, Nitrogen Ammonium (N -ammonium) BSS 3587, Nitrogen nitrate (N- nitrate) by BSS EN ISO 10304-2, Sulfates (SO_4) by BSS EN ISO 10304-2, Calcium Oxide (CaO) by EPA 6010C, Magnesium Oxide (MgO) by EPA 6010C, Potassium (K) by EPA 6010C, P (Phosphorus) by EPA 6010C, pH (H_2O) by BSS EN 12176 and heavy metals – arsenic (As), Cadmium (Cd), Chromium (Cr), Copper (Cu), Mercury (Hg), Nickel (Ni), Lead (Pd), Zinc (Zn) by EPA 6010C.

Table 1. Types of organic waste at different storage technologies

№	Identification
	Fresh manure
1	Cattle (liquid) manure obtained by water cleaning of the premises
2	Cattle (hard) manure produced with the participation of bed of straw
3	Pork (liquid) manure obtained in water cleaning of the premises
4	Pork (hard) manure after separation, obtained after dividing of manure fraction
5	Birds (hard)dry manure obtained by farming of chickens on bedding
6	Birds (hard) manure
	Stayed manure
7	Cattle (liquid) manure obtained by water cleaning of the premises - stayed 6-8 months
8	Cattle (hard) manure produced with the participation of bed of straw – stayed 6-8 months
9	Pork (liquid) manure obtained in water cleaning of the premises - stayed 6-8 months
10	Pork (hard) with a mixture of straw and sawdust– stayed 6-8 months
11	Birds (hard)dry manure obtained by farming of chickens on bedding
12	Birds (hard) manure - stayed 6-8 months
	Waste from wastewater treatment plan
13	Primary sludge
14	Secondary sludge
15	Mixed sludge dewatered by tape filter press
16	Mixed sludge (Stayed)
17	Methane tank-entrance
18	Methane tank- exit

Results and Discussion

The Table 2 presents the composition of different types of fertilizer (beef, poultry and pork) under different storage technologies as well as that of sediments from TP. For the primary indicator - Solids shows that the conservation process influenced the dry matter content for all types of fertilizer (beef, poultry and pork), regardless of the state - liquid or solid. With 1% tolerance exception substrate 11 overripe poultry manure to fresh Hard poultry manure substrate 5. Following the result of anaerobic digestion and fermentation processes ongoing loss of dry matter in standing fertilizers stayed 6 – 8 month fresh is to from 2.4% to 7.10% while the bovine solid manure they are the greatest. In terms of indicators of organic matter calculated on BOD variation is large between fresh and stayed fertilizers, the overall trend is towards a reduction of organic matter on standing manure, regardless of its type. Data for indicators of organic carbon correspond with those of the organic matter, while fresh manure carbon moves from 38,37 for pig manure to 25.25% and beef solid fertilizer on standing fertilizers, regardless of their tendency towards reduction of carbon.

Table2. Composition of organic waste in variety of storage technologies

№	DM, %	OM,% *	C, %	N,%	C:N	pH
1	6,68±0,19	44,82±0,47	35,98±0,29	4,61±0,09	5,61	8,66±0,36
2	59,30±0,76	28,43±0,13	25,25±0,98	0,99±0,01	25,51	7,76±0,13
3	4,04±0,28	33,33±0,93	26,19±0,76	7,14±0,21	3,67	7,86±0,14
4	37,46±1,13	40,80±0,11	38,37±0,65	2,43±0,06	15,79	7,36±0,09
5	48,16±0,03	44,85±1,11	38,03±0,23	6,48±0,11	5,87	8,46±0,19
6	50,50±0,01	36,00±0,27	32,38±0,91	3,62±0,13	8,94	8,35±0,24
7	4,04±0,04	43,37±0,31	30,78±0,04	6,76±0,06	4,55	8,54±0,15
8	52,20±1,02	35,21±0,23	31,92±0,19	1,25±0,02	25,54	7,46±0,11
9	1,61±0,01	39,62±0,27	36,31±0,09	3,31±0,13	10,96	7,95±0,24
10	40,47±0,28	37,07±0,93	31,92±0,76	5,09±0,21	6,27	8,24±0,14
11	49,05±1,35	37,93±0,35	26,84±0,17	4,12±0,08	6,51	8,27±0,07
12	44,59±1,35	26,99±0,35	22,87±0,17	4,78±0,08	4,78	8,47±0,07
13	4,90±0,09	29,12±0,22	28,61±0,11	0,51±0,03	55,90	5,53±0,06
14	0,90±0,01	30,18±0,14	25,37±0,31	4,81±0,78	5,27	7,52±0,09
15	41,81±0,98	30,79±0,18	28,08±0,14	2,71±0,10	10,36	7,56±0,19
16	56,46±1,13	15,60±0,11	14,50±0,19	1,14±0,05	12,72	6,63±0,11
17	2,96±0,15	35,52±1,01	30,59±0,22	4,93±0,69	6,20	7,22±0,07
18	2,20±0,03	20,58±0,20	18,48±0,31	2,10±0,27	8,80	8,60±0,16

*Organic matter is determined on the basis BOD

The percentages of reduction of the organic matter are shown in Table 5.

It is evident that the percentage of reduction of the organic compounds is higher in solid form fertilizers than in the liquid ones. Similar trends are observed in the data for mineralization of organic matter (Table 5), where the percentage of mineralization of the organic matter is highest in cow manure presented in both states – liquid and solid. Data from members of the collective from previous studies support the obtained results that cow manure is a suitable material for the production of biofuel. Table 5 clearly shows, however, that in manure from ruminants, regardless of the state (liquid or solid), the percentage of nitrogen (expressed as % from the DM – dry matter) is highest compared to other fertilizers. A tendency toward higher values is observed too in the mixture of bird manure with straw and filings.

Table 3. Contents of biogenic macro elements in organic waste

Indicators №	N, mg(kg ammoniu m	N,mg(kg nitrate	K,%	P,%	CaO %	MgO,%	Sulfates (SO_4,%
1	1,45	<6	3,11	3,14	2,93	1,35	0,21
2	0,14	<5	0,73	0,29	1,73	0,46	0,07
3	3,59	<11	4,20	1,28	3,63	1,13	0,12
4	0,13	<6	0,25	0,85	2,54	0,84	0,10
5	1,04	<4	2,04	0,98	2,74	0,78	1,12
6	0,72	<5	1,18	0,83	4,15	0,54	0,16
7	2,42	<11	3,34	0,99	4,00	0,99	0,43
8	0,02	177,8	0,68	0,49	1,42	0,62	0,03
9	2,38	27,8	0,68	3,33	5,19	3,94	0,10
10	4,76	<50	4,19	0,90	3,55	2,55	4,70
11	0,75	<5	1,30	0,52	6,31	0,66	0,12
12	0,26	382,5	0,39	5,48	9,07	3,57	0,48
13	2,354	-	0,50	1,95	4,90	1,12	-
14	2,418	-	1,05	4,10	2,59	1,04	-
15	5,844	-	1,36	8,61	1,76	0,31	-
16	<50	-	2,84	7,20	4,37	0,77	-
17	6,708	-	0,94	5,13	9,24	2,02	-
18	4,011	-	1,56	5,10	2,90	1,90	0,08

Table 3 presents some biogenic macro-elements in the different types of manure, stored in different technologies, as well as the composition of the residues in various stages of wastewater treatment. As can be seen, in fresh manure, regardless of the type, the content of the main biogenic macro-elements (with a few exceptions) is lower than the content in stagnant manure. Due to partial emission of carbon, oxygen and hydrogen (which are included in the biogas), the products of decomposition of organic matter have redistributed the studied macro-elements and correspondingly have resulted in higher levels in stagnant manure. In all three types of manure it has been found an increase in the amount of studied macro-elements. Our results for the composition of the different types of manure, obtained by different storage technologies, are a source of useful agro-ecology information. Having under consideration that C, N and O are not limiting to the plants, it can be noted that the values obtained on stagnant manures, in respect of the quantity of the tested macro-elements (nitrogen, phosphorus, potassium, calcium and magnesium

oxide), have high agrochemical value due to the relatively greater quantity of limiting macro-elements – nitrogen, phosphorus, potassium. With regard to the pH variation between the different manures from different storage methods, they are negligible (with 1% variation).

Table 4. Content of heavy metals

Components,mg(kg №	As	Cd	Cr	Cu	Hg	Ni	Pb	Zn
1	<5	<0,4	3,88	57,98	<1	5,58	<10	258,1
2	<5	<0,4	4,02	10,72	<1	4,33	<10	38,33
3	<5	<0,4	6,33	183,7	<1	8,56	<10	1815
4	<5	<0,4	5,37	63,96	<1	9,26	<10	1292
5	<5	<0,4	5,62	61,16	<1	7,25	<10	352
6	<5	<0,4	6,97	33,55	<1	3,30	<10	205
7	<5	<0,4	4,41	105,35	<1	6,47	<10	397,2
8	<5	<0,4	2,06	21,93	<1	2,13	<10	121,2
9	<5	<0,4	9,81	419,4	<1	13,47	<10	1401
10	<5	<0,4	13,17	25,34	<1	10,00	<10	115,6
11	<5	<0,4	3,86	34,65	<1	3,20	<10	229,8
12	<5	<0,4	8,81	183,26	<1	7,27	<10	1068
13	19,6	4,7	83,2	494,1	<1	41,3	97,0	1546
14	<5	3,6	95,5	308,0	<1	41,8	41,0	948
15	<5	1,0	22,3	114,8	<1	10,80	24.60	385,1
16	8,9	2,6	57,6	297,5	<1	25,8	77,7	894
17	<5	6,1	174,0	778,8	<1	85,05	159,1	2588
18	4	29	448	417	3	156	47	2454

In table 2 we are presented with the data on the composition of residues obtained as a waste product during the various stages of wastewater purification. The data show that the primary and secondary residues have lower values of DM (dry matter - 5.90 and 0.90% respectively) in comparison to the mixed residue. The low values of dry matter in the secondary residue compared to the primary residue (stage one of wastewater purification) are also noticeable. Regarding the organic matter indicator there aren't significant differences between the different residues. The method of storage has had an effect on the amount of organic matter in the mixed residues. In the stagnant mixed residue the amount of organic matter has decreased by almost half. This decrease is confirmed also in regard to the nitrogen and carbon content, despite that their ratio (C:N) values are too close with a tendency for higher ones in the stagnant one. The percentage of reduction of organic matter in the primary residue is highest (substrate 13-16). A similar tendency is observed when comparing the primary with the mixed residue which was dehydrated beforehand (Table 5). The percentage of mineralization of the

organic matter in the different types of residue is within 46%-54%. The high level of mineralization in the freshly dried residue compared to the primary one makes an impression. The obtained organic waste (substrate 17 and 18) is characterised by a composition and proportion which are analogous to the others during the various stages of wastewater purification. It has been noted, however, that substrate 17 (methane tank-input) has the highest percentage of nitrogen in dry matter. Similar to our results have been reported by *Marinova (2008).*

Table 5. Basic technology issues depending on the method of storage

substrates №	DM,% Fresh	DM,% Stayed	OB,% Fresh	OB,% Stayed	% of reduction of BOD (Fresh-Stayed)	% of mineralization of OM	% of N in fresh, % from DM
1 - 7	6,68	4,04	44,82	43,37	60,48	96,76	3,07
2 – 8	59,30	52,20	28,43	35,21	88,03	123,84	5,87
3 – 9	4,04	1, 61	33,33	39,62	39,85	97,87	2,88
4 – 10	37,46	40,47	40,80	37,07	108,03	90,85	2,06
5 – 11	48,16	49,05	44,85	37,93	102,00	84,57	3,12
6 – 12	50,50	44,59	36,00	27,65	88,29	76,81	1,83
13 - 16*	4,90	56,46	29,12	15,60	115,2	53,57	2,50
14 - 16*	0,90	56,46	30,18	15,60	62,27	51,69	6,50
13 - 15*	4,90	41,81	29,12	30,79	85,33	105,70	2,50
14 – 15*	0,90	41,81	30,18	30,79	46,46	46,46	4,33
17 - 18	2,96	2,20	35,52	20,58	74,32	57,94	14,59

*Fresh sludge and compost (mixed sludge out)

For an agro-ecological assessment of the analysed substrates, obtained at different stages of wastewater purification and stored as compost – freshly dried and as stagnant (substrate 15-16), the dynamics of the water-soluble fractions of the macro-nutrients, which are available to the plants, are of great importance. For this reason a leading indicator is the nutrients' content, while the water-soluble forms should be analysed as a criterion for the effectiveness of modelling the decomposition(decomposers' unit (modelling the unit of decomposition) in order to obtain an accurate dosage in soil application. Soil fertility and soil structure are determined by two main groups of products: nutrients in mineralized form and nutrients as organic compounds. In this respect the compost has an advantage as it retains all macro and micronutrients in two agro-chemically important categories: available mineral salts and the same elements in the form of organic compounds.

The movement of the toxic elements in the various types of manure, depending on the method of storage and those in wastewater at a various stage of their purification, are presented in Table 4. Their choice is based on the requirements in Ordinance 22(2001 on organic production in crop production. The

analysis of the obtained results shows that the content of arsenic, cadmium, mercury and lead are within the permissible values for the three types of manure in the different storage technologies. Essential in assessing the quantity of toxic elements is their impact on the soil at application and their potential for bioaccumulation along the trophic chain. In this respect it is important to consider the high values we obtained for chromium and nickel in the three types of manure. Pig manure has the highest chromium content (stagnant-liquid) – 9,81 mg(kg. Pig manure (fresh and liquid) and bird manure (fresh and solid) have similar chromium content, 6.33 and 6.97 mg(kg respectively. The lowest chromium content (approaching the permissible values) is in stagnant cow and pig manure (2.06 mg respectively). Nickel is also highest in pig manure (stagnant-liquid) – 13.47mg (kg and again it is the lowest in stagnant (dry) cow and pig manure (2.13 mg(kg respectively). The high levels of zinc in the fresh manure, stored in liquid or solid form makes an impression, as well as those in stagnant liquid pig manure. The deviations of the obtained values for pig manure compared to the other types of manure are in some cases almost 10 times. The levels of zinc are lowest in fresh cow manure, stored in liquid or solid state (25.12 and 38.33 mg(kg respectively). Stagnant (liquid) pig manure has the highest content of the microelement copper (419.4 mg(kg).

The results from the analysis show that the different types of manure, using different storing technologies, as well as the compost of the wastewater of the (Wastewater Treatment Plant) meet the requirements of Ordinance № 22 and EU Directive 2001(18 as sources of nutrients. Redistribution of some toxic elements depending on the method of storage was found, but their increased number in some organic waste is not a reason for them to be excluded as means to increase soil fertility.

Conclusion

The results obtained for the composition of the different types of manure (cow, pig and bird manure), stored using different technologies, as well as the organic waste (residues from wastewater) show:

- Variation of the percentage of dry matter, depending on the storage technology and the stage of wastewater purification.

- The method of storage (fresh and stagnant) has had a significant impact on the carbon content in the different types of manure and residues of wastewater.

- High levels of reduction of the organic matter, expressed in a per cent of the dry matter is observed, as well as high percentage of mineralization of the organic matter in stagnant manure and bioshlam obtained from the wastewater purification plants.

- The variations in the carbon and nitrogen content in the different manures and technologies of storage, affect the indicator C:N, which has close to the recommended optimal values for biofuel production in the three types of manure stored in a solid form.

- The storage technology in the different types of manure, regardless of their form (solid or liquid) and the bioshlam obtained from the wastewater of Sofia's Wastewater Treatment Plant, has not influenced the heavy metal content. All values are within the critically permissible as stated by Ordinance 35 for organic farming.

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Agroekološka vrednost organskog otpada u različitim tehnologijama skladištenja

B. Zaharinov Z. Shindarska, S.Marinova - Garvanska, V. Kirov, B.Baikov

Rezime

Studija je sprovedena na osnovu tri vrste đubriva (stajnjaka od krava, svinja i živine) i biološkog otpada koji se skladište u različitim tehnologijama, kako bi se uradila agroekolška procena.

Različite vrste đubriva i otpadnih voda u različitim tehnologijama za skladištenje analizirane su na osnovu sledećih parametara: suve materije (SM), organske materije (OM), organskog ugljenika (OC), Kjeldahlov Azot (N), amonijačni azot (N - amonijum), azot nitrat (N-nitrat), sulfati (SO4), kalcijum-

oksid (CaO), magnezijum oksid (MgO), kalijum (K), P (fosfor), pH (H2O) i teški metali - arsen (As) , kadmijum (Cd), hrom (Cr), bakar (Cu), živa (Hg), nikl (Ni), olovo (Pd), cink (Zn). Dobijeni rezultati pokazuju: varijaciju na procenat suve materije od 92,12% do 4,03% prema tehnologiji skladištenja. Način odlaganja nema suštinski uticaj na sadržaj ugljenika u različitim stabilnim đubrivima, gde svinjski stajnjak ima najveću vrednost azota u svim tehnologijama skladištenje. Tehnologija skladištenja različitih vrsta đubriva nema uticaja na sadržaj teških metala. Izuzetak je sadržaj cinka u ustajaloj (tečnoj osoki iz objekata gde se drže svinje). Visok sadržaj suve materije može se registrovati u otpadnim materijama iz stanica za prečišćavanje. U oba načina proizvodnje varijacije u sadržaju suve materije odnos biogenih makroelemenata C:N ostaje nepromenjen.

Slobodni oblici azota (N-amonijum i N-nitrat) u otpadnim materijama su veći u odnosu na drugi organski otpad u obe tehnologije skladištenja. Nema vrednosti preko kritički prihvatljivih za optadni materijal u obe tehnologije proizvodnje.

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BARRIERES IN TRANSGENES APPLICATION IN CONTEMPORARY LIVESTOCK PRODUCTION

M. Stupar[1], V. Vidović[1], D. Lukač[1]

[1]University of Novi Sad, Faculty of Agriculture, Department of Animal Science, Trg Dositeja Obradovića 8, 21 000 Novi Sad, Republic of Serbia
Corresponding author: milanko.stupar@laposte.net
Review article

Abstract: Efficiency of the genetic engineering application in contemporary livestock production is very low, actually only 1%. The main reason for failure lies in the fact that so far, little attention was paid to gene position effects, as well as fidelity of the numerous DNA polymerases active in eukaryotic cells. Any change in the DNA sequences, through insertions, deletions or nucleotide mutation, affects stability of the genome. This is particularly felt in DNA replication, where the information's transfer to the offspring calls into the question. It is necessary to find new approach to overcome these barriers. Is there are unique way to solve those two barriers. In recent years in the use are Sleeping Beauty transposase, Zn-finger nuclease, lentiviral integrase, site-specific recombinase, etc. Perhaps the solution lies in introducing the transgene into predetermined genome loci via site specific phage ϕC31 integrase into pronucleus of the target animal. In the context of transgenesis is better to talk about the bioDNA, corresponding to host DNA, and labDNA, DNA artificially constructed in laboratory.

Key words: gene position effect, genome integrity, transposase, integrase, Zn-finger nuclease, recombinase.

Introduction

Two sides are present in different modification of genome: foreign DNA and host genome. In this context, is better to talk about the bioDNA, which corresponds to the naturally evolved deoxyribonucleic acid (host DNA), and labDNA, which corresponds to the artificially DNA constructed in the laboratory. What can be done, and which constructs are allowed that labDNA survived and continued in the next generations? Foreign DNA (labDNA), like any other DNA, holds a vital energy that should be incorporated into a genome, otherwise is doomed. It seeks a place and a way to incorporate in order to replicate, transfer

their material to their offspring, no matter what the stranger is in the domestic system. It will be no surprise if the success of genetic engineering is, among other things, depended on the "adaptive evolution" of the labDNA. The capacity is enormous, the best examples are network-like mode of RNA virus evolution, i. e. adaptation to new conditions. There are novel virus genome develop by recombination between unrelated groups of RNA and DNA viruses (*Diemer, 2012*).

Homologous recombination could be used to specifically modify genes in mammalian cells (*Thomas and Cappecchi, 1987; Smithies, 2001*.). *Evans (2001)* was discovered that chromosomally normal cell cultures could be established directly from early mouse embryos. These cells are now referred as embryonic stem (ES) cells. All genes present in any genome may be accessible to modification by homologous recombination. Genes could be targeted in cultured cells and the targeted cells are, in most cases, embryonic stem cells (ESC). These two facts should be connected together, with one hand genetic homologous recombination, on the other hand ES cells. All the pieces were at hand to begin generating gene targeted embryonic stem cells.

For insertion of foreign DNA, gene position effect and genome integrity are barriers for proper expression efficiency of interested transgene. Predominant methods used to produce lab-animals have several limitations: genome integrity, insertion site, and copy number of the transgene cannot be controlled. Single-copy transgenesis can be expected with retroviruses, and transposons, but the transgene is integrated throughout the genome. One of the best methods in use is site-directed recombinase, which can span both of these obstacles.

Gene position effect

Gene position effect and faithful preservation of genome integrity are the huge obstacle to the gene targeting success. Numerous attempts have been applied to overcome these biological barriers. In eukaryotes a considerable proportion of the genome is represented by heterochromatin. The gene position effect is reflected in gene rearrangements, translocations, as a result of such changes gene may be integrated into chromosomes active zones (euchromatin) to the inactive zones (heterochromatin) and become inactive. There are constitutive and facultative heterochromatins. Constitutiv heterochromatin is predominantly positioned in pericentromeric and telomeric regions which is rich in repetitive sequences predominantly consisting in transposable elements. Facultativ heterochromatin represents transiently condensed and silenced euchromatin. One of the examples are the inactivated X chromosome in female mammals. The gene position effect may cause disruption in the activity of several genes close to heterochromatin, the

influence of heterochromatin is always in the direction of the nearest euchromatic gene.This means that it is very important to choose the phase of synchronized cells division for insertion of the gene of interest. Gene is not necessarily silenced by the effect of heterochromatin, because the heterochromatin did not spread across this gene early in development, when heterochromatin firs formed. It means that state of transcriptional activity of gene is inherited, once determined by its chromatin packaging in the early embryo.

Genes can be integrated/transferred from the chromosome active zones to the inactive zone and become silenced, and vice versa. The reversibility of the position effect demonstrates that a given genetic change is due to the position effect rather than to genetic mutation. The heterochromatin is activated upon being transferred to the euchromatin and becomes cytologically indistinguishable from the latter. Gene position effect describes also the variation of expression pattern exhibited by identical transgenes inserted in different sequences of DNA. The difference in expression is due to the neighboring enhancers. Each transgenic organism has the potential for a unique expression pattern, since each transgene has a different location in the genome. In mammals, the insertion of the transgenes can trigger transcriptional silencing of the transgene in order to protect the structure of host chromosomes.

As a result of their placement into the neighborhood of heterochromatin by chromosomal rearrangements, euchromatic genes become transcriptionally silenced. Studies with hsp26 transgene is a good example of gene silencing by heterochromatization. There are significant changes in the nuclosomal array from open configuration to regular pattern when the transgene (hsp26) is silenced by heterochromatisation (*Wallrath, 1995).* Different epigenetic factors are of vital influence on labDNA expression from gene position effect view. One of the most important is a transcriptional regulation by the nucleosomal remodeling. Cell uses multiple ways to avoid nucleosome repression of the transcription. Replication partially disrupts preexisting nucleosomes and creates new DNA what provides opportunity for *de novo* binding of transcription factors prior to the assembly of new nucleosomes. *(Krude, 1991; Randall, 1992; Gasser, 1996).*

Posibility to overcome GPE

Transpososase

DNA transposons are naturally occurring mobile genetic elements that “copy and paste” – class I and “cut and paste” – class II, themselves to move from one genomic location to another unique site within the host genome. Movement of DNA segments resulting in rearrangement of genomic DNA, initiates when

transposase forms a dimeric DNA-protein synaptic complex with transposon DNA end sequences. A transposon-encoded transposase recognizes the inverted terminal repeats flanking a transposon and catalyses the transposition of the element into the genome. Transposons are found in many major branches of life. They may have originated in the last universal common ancestor, or arisen independently. While some transposons may confer benefits on their hosts, most are regarded as selfish DNA parasites. Cell defend against the proliferation of transposons by piRNAs (piwi-interacting), siRNAs (small interfering) (*Wei-Jen, 2008*) which silence transposons after they have been transcribed.

Chicken primordial germ cells, for example, resist deliberate genetic modification, likely by silencing the introduced genes in the genome. Selection for transgene integration into chicken primordial germ cells (PGC) genome and sequencing of the insertion sites revealed that the transgenes preferentially inserted into active promoter regions, implying that silencing prohibited recovery of insertion in other regions. This is one of the interesting way to cell to liberate from the transgene. Despite the evidence for transcriptional silencing in PGCs, gene targeting of a nonexpressed gene was also achieved. Genetically modified chickens serve as models for studying developmental biology, as bioreactors for therapeutic products, as a model for disease resistance to enhance agricultural production. Results form study of *Mcdonald (2012)* shown that PGCs can be manipulated efficiently using transposon vectors. They used piggyBac and Tol2 transposons to modify PGCs stably. Tol2 transposon was five times more efficient than the piggyBac transposon in modifying chicken PGCs. *Mcdonald et al.(2012)* was shown that, contrary to the others, insulator DNA, sequences that shield regions of DNA form epigenetic silencing, were not required in the integrated transposon for transgene expression. PGCs containing integrated transgene were able to colonize the gonad of host embryos and form functional gametes that produce transgenic offspring. This transgenic chicken should become important contributors to health, science, and agriculture.

The article of *Dafa'alla (2006)* addresses the question about the behavior of transgenic animals in the wild population. Nonautonomous transposon insertions can be remobilized by exposure to a wild population transposase, when transgenic insects are release in environment. A method was developed to stabilized transposon insertion through post-integration excision of one end of the transposon. For this purpose, they used piggyBac transposase which does not necessarily use available pair of suitable terminal sequences. To generate transposon-free insertion, composite element with central domain flanked by two short nonautonomous piggyBac elements are used. The resulting insertions lack transposon sequences and are therefore impervious to transposase activity.

Sleeping beauty transposase

Sleeping Beauty (SB) transposon system is a synthetic DNA transposon that was constructed to introduce precisely defined DNA sequences into the animal genome. SB transposase inserts a transposon into TA dinucleotide base pair in a host genome. In the process of integration TA site is duplicated, and this duplication is a hallmark of transposition. All of the transposon identified in the mammalian genomes are non-autonomous because the transposase gene are non-functional and unable to generate a enzyme that can mobilized the transposon. This means that the host cell possess the mechanism that regulates activity of transposase.

The reconstruction of SB transposase was based on the concept that there was a primordial transposase genes found in fish that have been inactive more than 10 million years due to the accumulated mutations. A putative ancestral consensus sequence was predicted, and over the decade SB construct was increased which contains all of the motifs required for function *(Ivics, 1997)*. SB transposon can be use to carry a tansgene and associated elements that confer transcription regulation for expression at a desired level in specific tissue. SB can be used to discover the new gene function, to delivering DNA sequences this way, that gene is "knocked out". SB transposons combine the advantages of viruses and plasmids.

The use of non-viral vectors avoids some of the defenses that cells evolved against vectors. There are good few problems with most methods for delivering DNA to the genome using plasmids. Uptake of plasmid into cells is difficult, expression of transgenes from plasmid is brief due to cellular response that influence expression, it should be avoid multiple integrations which results in switch off expression of transgenes, using plasmids is much less efficient than using viruses. Using SB can provide useful levels of success of expressing transgenes for entire animals.

The long-term stability of labDNA insertion can be tested when insects, for example, are released in the environment. Population in wild might contamine the laboratory organisms with exogenous transposase insertion and remobilized transposon. Dafa'alla et al. was stabilized transposon insertion through post-integration excision of all transposon sequences from the labDNA, rendering it as inert to transposase as any other bioDNA. Hoping that such a approach may permit genetically modified insects to resist to natural selection.

Lentiviral integrase

In recent years lentiviral vector application have received a great attention including gene therapy, generation of lab-animals, and the stable dalivery of RNA interference molecules. The main reason for this are the qualities that lentivirus possess: - efficiently transduction of nondividing cells, ignoring the role of

replication, - shuttle large genetic cargo, long labDNA, - maintain stable long-term labDNA expression. A retroviral vestor system based on the HI-viruses was developed that could mediate stable *in vivo* gene transfer into many cells types. So far brain, liver, muscle, hematopoetic stem cells, terminally differentiated neurons, have been successfully transduced with lentiviral vectors carrying a variety of genes (*Naldini, 1996; Li, 2005*)

The HIV-1 proteins matrix, Vrp, inegrase are responsible for viral genome import in non-dividing cells. But, a sequence within *pol* gene, containing structural elements associated with the progress of reverse transcription, is also required for gene transfer by lentiviruses (*Follenzi, 2000)*. Once you have all this cargo loading into cell it is only matter of time when these lentiviruses will turn into viral diseases. Even when Follenzi state: „Full rescue of this step in lentivirus-based vectors improves performance for gene-therapy application". We should not lose sight of the resourcefulness of the viruses (*Diemer 2012*). When retroviruses integrate in the bioDNA they can cause insertional mutagenesis. The consequences depends entirely on the location within the bioDNA where viral genome is inserted; either within the gene, promoter region, repressor gene, enhancer; leading to altered cellular activity. This integration can be avoided using defective viruses, leaving their genome like episomes, free in the nucleus.

A key challenge for labDNA based on retroviral and lentiviral vectors is to minimize insertional mutagenesis. *In vitro* studies have shown that integration-deficient lentiviral vectors can mediate stable transduction. Integrase bounds to the attachment (att) region of the LTR to catalyze the covalent linkage with cellular DNA. Nearly half of the proviral DNA becomes episomal; the two major circular episomal forms results from nonhomologous end-joining and homologous replication, this is a potential for use of non integrated lentiviruses. The hot spots where it is applicable to modify LV can be seen in viral pol gene (integrase), and LTR regions. Viral *cis*-acting DNA elements, central polypurine tract sequence (cppt) and the woodchuck postregulatory element (WPRE), are included in efficiency of transduction. The cppt is a small DNA fragment in the pol gene usually cloned 5' to the internal promoter region, whereas the WPRE is cloned 3' to the inserted labDNA so that is in close proximity to the poly(A) strech in the 3'-LTR (*Park, 2007*). Another important LV transfer plasmid is a 400 bp deletion in the U3 region of the 3'-LTR, which deliberates 5'-LTR RNA pol II promoter activity following integration (*Park and Kay, 2001*.). From the other side, there are LTR sequences as 5'-LTR which acts like RNA pol II promoter, 3'-LTR acts to terminate transcription and promote polyadenilation, and the LTR sequence that recognizes sequence in bioDNA is necessary for integration.

Changes in LTR or in pol gene by introducing combinations of mutations may disable integrase protein itself or alter the integrase recognition sequence (att) in the viral LTR.

To overcome risk of insertional mutagenesis it is possible by developing a non integrative LV vectors. *Philippe et al. (2006)* are constructed LV vector with defective integrase by replacement of the 262 RRK motif by AAH. This derivative vector drives efficient labDNA expression in dividing and non dividing cells in vitro. They have estimated that the mutant vectors integrated 500-1250 times less frequently than wild type vectors, and it retains in episomal states. In that way LV vectors has great potential to overcome insertional mutagenesis, and be applicable in efficient labDNA transfer in bioDNA.

One of the interesting lentiviral vectors are simian immunodeficiency virus-based vectors. Nonhuman primates are appropriate for the study cognitive functions and brain disorders. But, human disease do not occur naturally in monkeys, therefore the transgenic animals are needed. In their experiment, *Yuyu et al. (2010)* produced four infant rhesus monkeys from four singleton pregnancies, of which two expressed EGFP (widely used) throughout the whole body. This is a very encouraging sign for the future use of lab-animals for gene therapy.

Genome integrity

Depending on the type of damage inflicted on the DNA's double helical structure, a variety of repair strategies have evolved to restore lost information. If possible, cells use the unmodified complementary strand of the DNA or the sister chromatid as a template to recover the original information. Without access to a template, cells use an error-prone recovery mechanism known as translesion synthesis as a last resort. There are 5 known prokaryotic family and 15 known eukaryotic types of DNA polymerases with different endo-, and exonuclease activity, participating in fidelity of DNA replication.
Damage to DNA alters the spatial configuration of the helix, and such alterations can be detected by the cell. Once damage is localized, specific DNA repair molecules bind at or near the site of damage, inducing other molecules to bind and form a complex that enables the actual repair to take place.

ncRNA and genome integrity

Some non-coding (nc) RNA are processed by DICER and DROSHA Rnase to give small double-stranded RNAs. Upon exogenous DNA influence, DNA-damage response (DDR) is activated at a single inducible DNA double-strand break (dsb). To repair this type of damage, DICER and DROSHA – dependent small RNAs (DDRNAs) are acting at genomic location of DNA break. Without DDRNAs cell is not alerted to DNA breaks and there are not respond to repair damage. Almost the entire genome is transcribed into RNA whose transcriptome is comprised of many low expressed non-coding RNA. All of these low expressed short RNA (20-25 nucleotides), contribute to regulate the functional organization

and expression of the genome, and like in the case of DDRNAs integrity of the genome.

To the monitoring of DNA, a new dimension is given by discovering short non-coding RNA (ncRNA) molecules that ensure the stability of the genome (*Francia et al., 2012*). So in addition to the water, and ncRNA contribute to the integrity of the genome. There are several classes of small RNA; micro RNA (miRNA), conventional small-interfering RNA (siRNA), and single stranded RNA (ssRNA). Regarding the genome integrity, two of them are of great importance, siRNA and ssRNA. siRNA of ~21 nucleotides are produced through defence against external nucleic acids. ssRNA are processed to ~27 nucleotides Piwi-interacting RNA (piRNA). It is probably that piRNA function as master controllers of transposable elements (transposons).

Possibility to overcome genome integrity
Zn-finger nuclease

ZFN is another arsenal that labDNA used to integrate in the bioDNA. ZFN are one of the most powerful and painless ways to change the structure of DNA without big loads on the integrity of the genome and positional effects of genes. ZFNs are artificial restriction enzymes, chimeras of a DNA-specific binding domain (Cys2His2 zinc-finger protein) and DNA-cleavage endonuclease FokI *(Kim, 1996.)*. The principle is that ZFNs induce site-specific double-strand break (dsb) in bioDNA that can be repaired by error-prone nonhomologous end joining (NHEJ) or by error-free homologous recombination (*Lombardo, 2007*). Introducing a dsb in eukaryotic genome stimulates DNA repair mechanisms. NHEJ can produce deletion or insertion of short sequences at the break *(Jeggo, 1998)*. Fok I restriction endonuclease from *Flavobacterium okeanokoites* consisting of N-terminal DNA-binding domain (5'-GGATG-3' : 3'-CATCC-5'), and C-terminal cleavage domain which cleaves the first strand 9 nucleotides downstream and the second strand 13 nucleotides upstream of the nearest nucleotide of recognition site (*Aggarwal, 1997; Wah, 1998*.). Each finger bind firstly 3 bp, the component sites are 9 bp in length, and the optimum for paired sites is an inverted orientation with a spacer of 6 bp (*Bibikova, 2001*). If all nucleotides in the mutated target are contacted specifically, these live 18 bp recognition sequence, long enough to be unique even in a complex genome. If ZFNs finds the recognition sequences, and if separation between the component 9-mers is not a 6 bp, than corresponding linker between the binding and cleavage domains should be added. ZFN approaches greatly facilitated the ability to direct mutations arbitrary to mutated sequences without the need to alter bioDNA in advance.

Site-specific recombinase

Since the initial discovery that recombinases can be used in genomic engineering (*Sauer, 1987*), the recombinase-mediated cassette exchange, one of the technology in the field of reverse genetics, is of increasing relevance. To effectively resolve complex labDNA insertion, and to avoid epigenetic influence, site-specific recombination technology enters the field. Site-specific recombinase are grouped into two families: the tyrosine recombinase (such as Cre, Flp), and serine recombinase (Tn3 resolvase, ϕC31, Bxb1, R4 integrase).
One of the best examples are the ϕC31 integrase in mice (*Tasić et al., 2010*). In their experiment, ϕC31 integrase (Fig.1.) were used to catalyze recombination between one or two attB sites in a labDNA with one or more tandem attP sites that they previously inserted into specific loci in mice bioDNA. Via pronuclear injection *Tasić et al. (2010)* received single-copy insert into predetermined chromosomal loci with high efficiency (up to 40%).

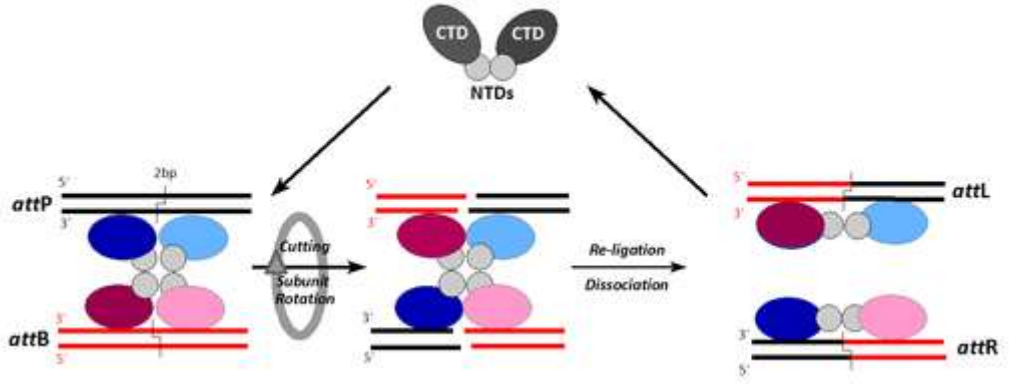

Fig. 1: Action of the ϕC31 integrase (from the ϕC31 phage), one of the Ser-recombinases. Subunit-rotation (180°) permits the exchange of strands while covalently linked to the protein partner. The intermediate exposure of double-strand breaks bears risks of triggering illegitimate recombination and thereby secondary reactions. Here, the synaptic complex arises from the association of pre-formed recombinase dimers with the respective target sites (CTD/NTD, C-/N-terminal domain). For Ser-recombinases, each site contains two arms, each accommodating one protomer. As both arms are structured slightly differently, the recombination pathway converts two different substrate sites (attP and attB) to site-hybrids (attL and attR) .This explains the irreversible nature of this particular recombination pathway, which can only be overcome by auxiliary "recombination directionality factors".

Conclusion

After many years of wandering in search of the best ways to make transgenic animals, perhaps we are on a track to achieve. Latest developments in molecular

biology have made it possible to apply new techniques in scoring labDNA, without major changes in the bioDNA. To overcome gene position effect, genome integrity, and copy number of the transgenes, in the application are the latest technologies. For targeting the gene of interest, perhaps different recombinase can be primarily used (Cre and Flp). To avoid any impact on the integrity of the genome, ZFNs and ϕC31 recombinase-integrase can be used in the future.

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EMERGING VECTOR BORNE DISEASES – RISK FOR PUBLIC HEALTH

S. Savić[1], B. Vidić[1], Ž.Grgić[1], A.Potkonjak[2], D. Sakač[3]

[1] Scientific Veterinary institute „Novi Sad“, Rumenački put 20, 21000 Novi Sad
[2] Department of veterinary medicine, Agricultural faculty, Trg Dositeja Obradovića 8, 21000 N. Sad
[3] Medical Faculty, University of Novi Sad, Hajduk Veljkova, 21000 Novi Sad
Corresponding author: sara@niv.ns.ac.rs

Abstract: In the past vector borne diseases represented a major public helath concern in most tropical and subtropical areas, but today they are an emerging threat for continental and developed countries. Vector borne zoonotic diseases occur when vectors, animal hosts, pathogens and susceptible human population exist at the same time, at the same place. Global climate change is predicted to lead to an increase in vector borne infectious diseases and disease outbreaks. It could affect the range and popultion of pathogens , host and vectors, transmission season, etc. Reliable surveilance for diseases that are most likely to emerge is required. It is expected from veterinarians to play a fudamental role in first of all prevention and then treatment of vector borne diseases in dogs. Canine vector borne diseases represent a complex group of diseases including anaplasmosis, babesiosis, bartonellosis, borreliosis, dirofilariosis, erlichiosis, leishmaniosis. Some of these diseases cause serious clinical symptoms in dogs and some of them have a zoonotic potential with an effect to public health. During a four year period, from 2009-2012, a total number of 236 dog samples were analysed for vector borne diseases (borreliosis, dirofilariosis and leishmaniasis) in routine laboratory work. The analysis were done by serological tests – ELISA for borreliosis, dirofilariosis and leishmaniasis and modified Knott test for dirofilariosis. This number of samples represented 52,91% of total number of samples that were sent for analysis for different infectious diseases in dogs. Annually, on avarege half of the samples brought to the laboratory to analysis for different infectious diseases are analysed for vector borne diseases. In the region of Vojvodina, the folowing vector borne infectious diseases have been found so far in dogs: borreliosis, dirofilariosis, leishmaniasis, erlichiosis and anaplasmosis.

Key words: vector borne diseases, public health, dog, ELISA

Introduction

In the past vector borne diseases represented a major public helath concern in most tropical and subtropical areas, but today they are an emerging threat for continental and developed countries. There are countries where environmental conditions are not so favorable to certain vector populations, but immigration allows them to persist (*Rascolau, 2012*). The complex epidemiology of vector-borne diseases creates significant chalenges in the design and delivery of prevention and control strategies, especialy in light of rapid social and environmental changes. Many diseases are spacially constrained, for example vector-borne and zoonotic diseases occur where and when vectors, animal hosts, pathogens and susceptible human populations overlap (*Hongoh et al, 2011*). Global climate changes is predicted to lead to an increase of infectious disease outbreaks. Reliable surveillance for diseass that are most likely to emerge is required. Climate changes could affect the range and population size of pathogens, hosts and vectors, the length of the transmission season and the timing and persistance of out breaks (*Greer and Fisman, 2008*). If there is an apperiance of infectious diseases that are considered to be eradicated from before or that are totaly under control, they are called emergent infectious diseases (*Morse 1991*). Emerging infectious diseses can be defined as infections that hve newly appeared in a population, or are rapidly increasing in incidence or geographic range. Many oof these diseases are zoonoses (*Morse, 2004*). From all the causative agents of emergent infectious diseases, 60-70% of them have a zoonotic potential (*Taylor et al., 2001*). Zoonozes are infectious diseases which can be transfered from animals (mamals) to humans. Vector borne zoonotic diseases occur when vectors, animal hosts, pathogens and susceptible human population exist at the same time, at the same place. Maps of expected distributions of vector existance are often presented as a risk to exposure to a pathogen. Global climate change is predicted to lead to an increase in vector borne infectious diseases and disease outbreaks. It could affect the range and popultion of pathogens , host and vectors, transmission season, etc. Reliable surveilance for diseases that are most likely to emerge is required.

The factors of emergence are the following: changes in ecology, changes in demography and human behaviour, changes and adaptations of microorganisms, improvement in technology and changes in industry, international transport and trade and incompliance of public health measures (*Stevanović et al, 2011*). Changes in ecosystem may lead to increase of population in natural hosts, or vectors for certain emergent infectious disease. These factors are becoming increasingly prevalent, suggesting that infections will coniniue o emerge and probably increase. Strategie for deaaling with this problem include focusing atention on promoting disease emergence, especially in situations when animals

and humanss are in contact and implementation of effective disease surveilance and control (*Morse, 2004*).

For practicing veterinarians, vector borne diseases represent a constant chalenge. The health of companion animals never played a more important role in a family life. It is expected from veterinarians to play a fudamental role in first of all prevention and then treatment of vector borne diseases in dogs. Canine vector borne diseases represent a complex group of diseases including anaplasmosis, babesiosis, bartonellosis, borreliosis, dirofilariosis, erlichiosis, leishmaniosis, rickettsiosis. Some of these diseases cause serious clinical symptoms in dogs and some of them have a zoonotic potential with an effect to public health.

A tick species I.ricinus is usuall predominant among ticks originateingform erbi and is one of the most widely distributed. A significant presencce of B.burgdorferi sensu lato was detected in I.ricinus ticks from Serbia , using dark field microscopy (*Milutinović et al, 2006; Milutinović and Radulović, 2002*).

Material and methods

During a four year period, from 2009-2012, a total number of 446 dog samples were analysed for different zoonotic diseases. For vector borne diseases (borreliosis, dirofilariosis and leishmaniasis) 236 dog blood samples were analysed in routine laboratory work, which makes 52,91% of total samples analysed for infectious diseases. The analysis were done by serological tests – ELISA for borreliosis (Microgen IgG and IgM and Euroclone comercial ELISA set kits for detection of specific antibodies against *Borrelia*), leishmaniosis (Euroclone comercial ELSA set kit for detection of specific antibodies against *Leishmania*) and dirofilariosis (IDEXX comercial ELISA set kit for detection of specific antibodies against *Dirofilria*). The procedure of analysis was done by the original manufacturer's instructions. Diagnostic of dirofilariosis was also done with modified Knott test for dirofilariosis. The samples were collected when dogs came for a routine check-up, or when they were sent for a certain disease detection because of the clinical simptoms that could be found in the animal. Some of the dogs had clinical symptoms which could be identified as characteristic for borreliosis, leishmaniosis or dirofilariosis. For borreliosis 117 dog blood samples were examined, for dirofilariosis 31 sample and the examined number of samples for leishmaniosis was 88.

Results and Discussion

The number of samples analysed for vector borne zoonozes: borreliosis, leishmaniosis and dirofilariosis represented 52% of total number of samples that

were sent for analysis for different infectious diseases. Annually, on avarege half of the samples brought to the laboratory to analysis for different infectious diseases are analysed for vector borne diseases. In the region of Vojvodina (northern part of Serbia), the folowing vector borne infectious diseases have been found so far in dogs: borreliosis, dirofilariosis, leishmaniasis, erlichiosis and anaplasmosis. For erlichiosis and anaplasmosis a routine diagnostic procedure started only in 2012, so the number of these samples was not taken ino the consideration during the study.

In the same region the folowing diseases have been diagnosed in humans, so far: borreliosis, dirofilariosis and leishmaniosis (imported cases). Borreliosis exsits as a common disesae in humans in Serbia. Several cases of dirofilariosis in humans have been found during the last four years in Vojvodina and also cases of leishmniosis have been found during the same period, but only as imported cases.

During the observed period (2009-2012), 117 dogs were examined for lyme borreliosis in routine work and 17 of them were found positive (14.53%). Seroprevalence for lyme borreliosis in dogs was studied previously, for the earlier three yaer period (2006-2008) in a larger number of samples, and in the same region and it was found to be 25.81%. Also, the prevalence of lyme borreliosis in ticks in the northern part of Serbia, was found to be 22.12%, depending on the region (*Savić et al, 2010*). In a study from Milutinovic et all, the highest prevalence rate found in Serbia among ticks for *B.burgdorferi sensu lato* was 42,5%. The presence of five *B.burgdorferi sensu lato* genospecies was found: *B. burgdorferi sensu stricto, B. afzelii, B. garinii, B. usitaniae and B. valaisiana*. Also co-infecions were found in ticks with *B. burgdorferi sensu lato* and *A. phagocitophilum* (*Milutinović et al., 2008*).

During the same period from 2009-2012, the number of 31 dogs were examined for dirofilariosis – with or without clinical symptoms, and in 16.1% the presence of microfilaria or specific antibodies against dirofilaria was detected. In the previous period a study was done on working and military dogs in the same region, where as a result a seroprevalence for dirofilariosis was found to be 18% (*Pajković et al, 2010*).

The number of exmined dog blood samples for leishmaniosis, during the period of study, was 88. The number of positive samples detected for leishmaniosis was 6, which is 6.81%. A certain number of dogs was taken abroad in the previous period and were examined upon return to the country (the number of examined dogs was 21) for the presence of specific antibodies against *Leishmania.* The dogs were taken abroad (Italy, Greece or Montenegro) for a certain period of time (1 week – several months). From the total of 21 samples, in 28.57% a positive findings for leishmaniosis was detected and some of the dogs had clinical symptoms of leishmaniosis (*Savić et al., 2012*).

Conclusion

Human and animal health is conected today into a One health concept, which focuses on zoonotic pathogens emerging from wild life, domestic animals and companion animals. A role of companion animals influence to public health is more important over the years, esspecially the major vector borne infectious diseases that are shared by humans and dog or cats. There should be an interaction between veterinary and human medicine for the benefit of domestic, wild animal and human health. It should always be in our minds that there is an interaction between human and domestic animal and wild life health with global zoonotic disease pandemcs and emerging infectious diseases which came from these animal species. From the total of 446 dogs examined for different infectious diseases, 236 of them were tested for vector borne diseases that exist in the region in dogs, vectors and humans. For one of the three studied diseases, in total 28 positiva samples were found, which is 6.28%.

Leishmniosis, borreliosis, erlichiosis, anaplasmosis, etc are considered as major vector borne infectious diseases that are shared by man and dogs, from the One health concept point of view. There should be an interaction between veterinar and human medcine, with clinitians, researchers and government working together for the benefit of domestic, wild animal and human health and the global environ ment (*Day, 2011*).

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Emergentne vektorske bolesti – rizik za javno zdravlje

S. Savić, B. Vidić, Ž.Grgić, A.Potkonjak, D. Sakač

Rezime

Nekada su vektor prenosive bolesti predstavljale veliki problem za javno zdravlje u većini tropskih i subtropskih zemalja, a danas predstavljaju emergentnu pretnju za kontinentalne i razvijene zemlje. Vektor prenosive zoonozne bolesti se javljaju kada se u isto vreme nađu na istom mestu vektori oboljenja, žvotinje domaćini, uzročnik i prijemčiva ljudska populacija. Globalne klimatske promene

utiču na porast vektor prenosivih infektivnih oboljenja i pojavu epidemija. Mogu da utiču na populaciju uzročnika, domaćina i vektora, sezonu prenošenja, itd. Potrebna je pouzdan nadzor nad bolestima za koje postoji najveća verovatnoća da se pojave.

Od veterinara se očekuje da preuzmu glavnu ulogu u najpre u prevenciji a onda i tokom terapije vektor prenosivih bolesti kod pasa. Vektor prenosive bolesti kod pasa predstavlja grupu oboljenja: anaplazmozu, babeziozu, bartonelozu, boreliozu, dirofilariozu, erlihiozu, lajšmaniozu. Neke od ovih bolesti izazivaju ozbiljne kliničke sipmtome kod pasa, a neke imaju i zoonotski karakter, a time i uticaj na javno zdravlje.

Tokom četvorogodišnjeg perioda od 2009.-2012. godine, pregledano je ukupno 236 uzoraka krvi pasa na vektor prenosive bolesti (boreliozu, dirofilariozu, i lajšmaniozu), tokom rutinskog dijagnostičkog rada. Analize su rađene serološkim testovima – ELISA za boreliozu, dirofilariozu i lajšmaniozu i modifikovan Knotov tst za dirofilariozu. Ovaj broj uzoraka koji je ispitavan na prisustvo vektor prenosivih bolesti je predstavljao 52,91% od ukupnog broja uzoraka pasa koji su poslati na ispitivanje na različite zarazne bolesti kod pasa. Tokom godine, u proseku polovina uzoraka koji se donesu na analizu na različite infektivne bolesti se pregledaju na vektor prenosive bolesti. Na području Vojvodine, sledeće vektor prenosive bolesti su dijagnostikovane do sada kod pasa: borelioza, dirofilarioza, lajšmanioza, erlihioza i anaplazmoza.

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THE FINDINGS OF SOME BEE PATHOGENS AND CCD SYNDROME

I. Stojanov, J. Petrović, M. Kapetanov, M. Živkov Baloš, J. Prodanov-Radulović

Scientific Veterinary Institute «Novi Sad», Rumencki put 20, Novi Sad, Republic of Serbia
Corresponding author: igor@niv.ns.ac.rs
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Abstract: Colony Collapse Disorder (CCD) is a syndrome characterized by the appearance of complex disease symptoms. Etiology of the disease is unclear and multi-causal. This syndrome causes a lot of damage in beekeeping which is important branch of agricultural production. Indirectly, crop, vegetable and fruit production could be hampered due to the reduction in the number of bee colonies and bees, which play an important role in plant pollination. Causes of CCD syndrome cannot be determined with certainty because multiple factors lead to the syndrome and present only a part of the joint action of different etiological factors. The presence of *Nosema spp*. and *Varroa destructor* in honey bee colonies are contributing to the development of the CCD syndrome. Their role in the decline of bee colonies is specific and it is therefore important to monitor its presence and abundance in apiaries. For these reasons, the subject of our work was to monitor the presence of *Nosema spp*. and *V. destructor* in order to determine its possible role in CCD syndrome in the field. Winter losses in apiaries in Serbia experiencing CCD syndrome during 2007/2008 amounted to 28.7%, 2008/2009. 21.3% and in 2009/2010 to 12.8%. According to the data obtained from bee associations, winter losses in 2012 amounted to about 15% of bee colonies. Despite the fact that in Vojvodina in recent years, the number of bee colonies that are suffering from CCD syndrome in the fall, it can be seen that, in some bee colonies, was affected up to 80% of hives. During the year 2012 the presence of *Nosema spp*. was just over 50% of the samples while *V. destructor* was found in 13,3% samples.

Keywords: *Nosema spp., Varroa destructor*, CCD syndrome

Introduction

The importance of bees for wildlife on the Earth can be seen by their direct effect on plants because pollination allows the reproduction of plants, whereas the indirect effect to the animal kingdom in relation to diet is also important including

humans (*Ellis and Delaplane,* 2008). This statement would best support the words of Alfred Einstein, "If the bee disappeared off the face of the Earth people would not have survived for more than four years" (*Stindl and Stindl Jr.,* 2010). For these reasons, any disorder related to the reduction in the presence of bees in nature can affect the wildlife on the Earth. In that respect it is important to understand the mechanisms that lead to them (*Plavša et al*. 2008). Syndrome of disappearing bee colonies (Colony Collapse Disorder CCD) is a disorder that appeared, according with the literature (*Vanengelsdorp et al.* 2009a) at the end of the nineteenth century in the United States (according to available documentation that described the clinical findings), but only at the end of the twentieth century, researchers started with considerable testing. The disorder is characterized by the sudden disappearance of worker bees, with a noticeable difference in the number of older bees (reduced number), in relation to brood and young bees. Syndrome (CCD) is characterized by the lack of dead bees that cannot be found in the hive as well as around it, and the absence of invasion by weak societies bee predators (primarily various types of insects) and cleptoparasitism by bees from neighboring colonies (*Vanengelsdorp et al.,* 2009a). Since it is a syndrome that encompasses a number of symptoms related to the same disorder, until now it has not, with some confidence, determined the precise etiologic factor leading to it. Syndrome is a multicausal and can be attached to one or more of the associated factors such as quantity and quality of pollen and protein (*Naug D.,* 2009), which the bees introduce to the hive, the presence of parasites (*Varroa*, *Acarapis*) (*Guzman-Novoa et al.,* 2012), pathogens (*Nosema*, viruses) (*Teixeira et al*., 2008), pesticide residues (*Vanengelsdorp et al,.* 2009a; *Garrido et al.,* 2013), bee genetics and morphological characteristics (*Vanengelsdorp et al.,* 2009a; *Stindl and Stindl Jr.* 2010; *Evans and Schwarz* 2011; *Vanengelsdorp, Meixner* 2010). For these reasons, the subject of our paper was to investigate the occurrence of *Nosema spp.* and *Varroa destructor* in the samples of bees delivered to the laboratory with the aim to determine is their presence in apiaries enrolled in CCD syndrome and to try to establish a link between this findings and data about the CCD syndrome in the field.

Material and Methods

During the year 2012 the laboratory received the samples of bees as a part of regular health control for the presence of varroa and a nosema, the samples originated from beehives which were, according to the observations of beekeepers and descriptions of the clinical condition, suspicious to the disappearing bee syndrome. The most important problems that have been described were a sudden weakening of the colonies in which there was enough food and broodnest or the

typical findings of disappearing bees without the presence of dead individual were noticed. The samples also included bees that beekeepers are able to collect from large amount of dead and /or poorly vital bees found in the hive or around them. During one year of the study for the presence of pathogens nosema 55 samples were processed while 75 samples were processed to detect the presence of pathogens varosis. From the bees that arrived for the examination for the presence of a nosema, the abdominal part was removed and macerated in mortar with pestle with the addition of small amounts of physiological saline. Confirmation of the presence of *Nosema spp*. was done under microscope (magnification 40 x 100) after the contents of the abdomen was transferred to the glass slides. Samples of live bees, which should be tested for the presence of *Varroa destructor*, were kept in the freezer for 1 hour after which they were placed on the paper. Intersegment parts of the abdomen was analyzed. Parasites that fired the bees were easily noticed by the white surface of the background. Each sample contained 50-100 of live bees (*Lolin et al.,* 1984). Anamnesticall data on the number of bee communities, which experience the disappearance syndrome were obtained from beekeepers' associations.

Results and Discussion

Information obtained from the beekeepers' associations indicate that the loss of bee colonies in Serbia were present in varying degrees. Losses in the winter 2007/2008 amounted up to 28.9% in 2008/2009 21.3% and 2009/2010 to 12.8%. During 2012 losses of bee colonies were at a level of 15%. Despite the total decreased number of affected colonies, in certain vanishing bee apiaries in Vojvodina the loss of 80% of the colonies was noted. Data from the literature suggest that in the United States of America (USA) in 2007, some beekeepers had losses of 80% - 100%, and the total loss of bee colonies in the winter 2006/2007 was 32%. The following winter the losses increased to 36% and in 2008/2009 were 29%. Total losses in the period 2007-2009 ranged from 36% to over 60% (S*tindl and Stindl Jr.* 2010). In the past, while the CCD sindrome was not explained well, the losses of colony were between 10-30%. Today, when CCD is well-defined the losses include 50-90% with significant clinical differences (Farooqui et al., 2013). In the U.S.A. (*Johnson* 2009) data indicate that the reduction in the number of bee colonies on the East Coast 2007/2008 was in the amount of 35.8%, while in 2008/2009 estimate of losses was 28.6%.

Test results of samples examined for the presence of pathogens *Nosema* (*Nosema spp*.) are presented in Table 1. The study included samples that arrived in one year and are divided into two groups depending on the season (spring and autumn). The values given in the table are determined in relation to the number of spores that

were present in bees. Experience of researchers indicates that severe infection may consider finding 20 million or more of *Nosema spp*. spores, while the low infection means findings below this number.

Table 1. Results of examination of bees on *Nosema spp*.

Value	Autumn	Spring	Total of 55 materials
+++	1	20	21
++	3	3	6
+	8	1	9
-	16	3	19

+++ = severe infestation, ++ = medium infestation, + = low infestation, - = not present

The materials were processed did not belong apiaries with established disappearing bee syndrome, that was not present clinical findings of this syndrome. The presence of *Nosema spp*. from the clinical aspect was asymptomatic. It may be noted that the intensity of infection was much higher during the spring season, when the sum of the maximum and medium infestation comprised almost 50% of the samples. In the autumn, the absence of a nosema or their presence in small numbers was recoreded. Data from the literature suggest that in some locations in Spain *Nosema spp*. was present 100% (*Fernández et al*. 2012), and analysis showed that the pathogen was widespread in the country. The same authors suggest that during the two years of monitoring 87% of bee colonies were without clinical signs, with normal production and development of biological characteristic of the apiaries. Molecular studies have shown that in the apiaries in Spain is the dominant *Nosema ceranae* (50-100%) in contrast to *Nosema. apis* (3% - 16.7%). Despite the presence of a high number of *N. ceranae* the symptoms of syndrome disappearing bee colonies was not found although this pathogen is mentioned in the literature as major factor that leads the development of the aforementioned syndromes (*Vanengelsdorp and Meixner* 2010; *Vanengelsdorp* 2009a; *Johnson* 2009). Examining the importance of interconnectedness *N.apis* and *N. ceranae* in the work of *Smart and Sheppard* 2012, it was suggested that their similarity is lower than previously assumed. The researchers found that the *N.ceranae* spreads between bees differently, and that in some cases the young bees were infected early, while on the other side some bees lived a whole life without infection with *N. ceranae*. Analysis of the presence of *N. apis* and *N.ceranae* in four different types of bees (*Chaimanee et al.* 2010) showed differences. *Apis melifera*, European (Western) bee was in 77.5% of cases positive for *N.ceranae*, while three species

characteristic of Asia *A.ceranae*, *A. florea*, *A. dorsata* were significantly less infected (22.2%, 45.4%, 37.5%) with this type of nosema.

The results of testing the presence of *Varroa destructor* are presented in Table 2. The tests were conducted over a period of one year. In the issued samples there were 50-100 live bees.

Table 2. Results of examination of bees on *Varroa destructor*

No. of Varroa per sample	Total examined of samples	Results
1-5	75	8
>5		2

Varroa destructor is considered one the most destructive parasites of bees widespread in nature (*Vanengelsdorp and Meixner,* 2010). This parasite is transmitted to Europe and settled in *Apis meliffera*, from Asia where genuine own hosted Asian bee *Apis ceranae*. This bee has spread *V. destructor* throughout the world and it is now in all places where beekeeping is based on *A. mellifera* (*Vanengelsdorp et al.,* 2009b; *Rosenkranz et al. 2010*). The importance of transferring *V. destrructor* from Asia to Europe is reflected in the *Apis ceranae* special characteristics that make it more resistant to parasites. Differences in resistance to *Varroa spp*. present between the Asian honeybees *Apis cearanae* and the European bee *Apis mellifera* were studied in work of *Zhang et al*. (2010). Authors concluded that the increased resistance of *A. ceranae* is related to the expression of genes that control metabolic processes (*Contzen et al*., 2004) and neural signals that influence susceptibility to the development of parasite larvae in closed broodnest. Our results show that the number of colonies infected with *V. destructor* is not large: total of 10 samples (13.3%) were infested with parasite. The our finding indicate that beekeepers use various pesticides (acaricides) in order to reduce the number of mites or eliminate them greatly. The problem is evidently linked to fast adaptation of *Varroa spp*., and rapid development of resistance to the applied agents (*Guzman-Novoa et al*., 2012). Synthetic acaricides based on organo posphoric compound, pyrethroids and amitraz that is used in the last 15 years have less effect on the mites because of parasit resistance (*Rosenkranz et al.,* 2010). Their negative impact is much more to be seen on bees because components of these products remain in the wax and other bee products. In addition to these "strong" acaricide today is widespread use of so-called "soft" acaricides such as organic acids and essential oils of plants (*Rosenkranz et al.,* 2010). The use of these products has its advantages because it does not lead to development parasit resistancy, there is no accumulation of products in bees and some of them (formic acid) can act on parasites in a closed broodnest. Undesirable traits are related to

dosage, because the border between a therapeutic dose and toxicity to bees is very narrow.

Conclusion

Studies have shown that nosemosis and varosis were found in beehives but their presence was not high. The presence of *Nosema spp*. even in materials that have a high percentage of spores was not accompanied by clinical symptoms that would suit syndrome of disappearing bee colonies. Similarly, the positive findings with *Varroa destructor* were not possible to connect with the characteristic symptoms of CCD and subsequently interrelationship with CCD was not confirmed.

Significant drawback of monitoring and control of honey bee colonies disappearing syndrome is associated with lack of well-organized data collection and the number and extent of occurrence of this syndrome. Analysis for the presence of pathogens, parasites, residues or other agents, alone or synergistically leading to the disappearance of the bees cannot perform well if they are organized by the owner of bees itself or beekeepers associations without experts and laboratories who establish good coordination with each other. The complexity of symptoms and etiological of complexity require establishment firm relationship in data collection and collaboration of experts from multidisciplinary areas, as would not realized the opportunity which Einstein suggested.

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Nalaz nekih pčelinjih patogena i ccd sindrom

I. Stojanov, J. Petrović, M. Kapetanov, M.Živkov Baloš, J. Prodanov-Radulović

Rezime

Colony collapse disorder (CCD) predstavlja sindrom koji se karakteriše pojavom složene slike većeg broja simptoma oboljenja i etiologijom koja je

nedovoljno jasna i multikauzalna. Sindrom nanosi, direknto, velike štete pčelarastvu kao jednoj od važnih grana poljoprivredne proizvodnje ali i indirektno pre svega ratarskoj, povrtarskoj i voćarskoj proizvodnji zbog smanjenja broja pčelinjih društava, odnosno pčela, koje igraju važnu ulogu u oprašivanju biljaka.

Uzrok nastajanju CCD sindrma je složen i ne može se sa sigurnošću odrediti da li je neki od faktora koji dovodi do razvoja sindroma primaran ili je samo deo udruženog delovanja različitih etioloških faktora. Prisustvo *Nosema spp*. i *Varroa destructor* u pčelinjim društvima doprinose razvoju CCD sindroma. Njihova uloga u slabljenju pčelinjih društava je specifična i zato je važno pratiti njihovo prisustvo i brojnost u pčelinjacima. Iz navedenih razloga predmet našeg rada je praćenje prisustva *Nosema spp.* i *V. destructor* u dostavljenim uzorcima pčela sa ciljem da se utvrdi kolika je njihovo zastupljenost u pčeljinjacima i da se pokuša uspostaviti veza između njihovog nalaza i podataka o prisistvu CCD sindroma na terenu.

U Republici Srbiji zimski gubici u pčelinjacima koji su po svojim karakteristikama odgovarali CCD sindromu tokom 2007/2008. iznosili su 28,7%, 2008/2009.(21,3%), a 2009/2010. (12,8%). Tokom 2012. godine prema podacima dobijenim od pčelarskih udruženja, zimski gubici su iznosili oko 15% pčelinjih društava. I pored činjenice da se procentualno smanjio broj zahvaćenih društava, nestajanje pčela u pojednim pčelinjacima u Vojvodini iznosilo je i do 80% društava. U 2012. *Nosema spp.* je u jakoj i srednjoj infestaciji bila zastupljena u nešto preko 50% ispitanih uzoraka, a *V. destructor* u 13,3%.

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PHYSICOCHEMICAL PARAMETERS OF BULGARIAN RAPE HONEY (*Brassica spp.*) AND CORIANDER HONEY (*Coriandrum sativum L.*)

R. Balkanska, M. Ignatova

Institute of Animal Science, Spirka "Pochivka" 1, 2232 Kostinbrod, Bulgaria
Corresponding author: r.balkanska@gmail.com
Original scientific paper

Abstract: Honey has been essentially used as a food all over the world since ancient times. Honey contains sugars, organic acids, enzymes, amino acids, mineral elements, pollen. The composition of honey varies greatly depending on the honey floral source. The purpose of this study was to verify and compare some physicochemical parameters of rape honey (*Brassica spp.*) and coriander honey (*Coriandrum sativum L.*) samples collected from Bulgaria and to increase the data for these unifloral honey types. Seven rape honey samples and six coriander honey samples were analyzed. The quality criteria of honey are specified by the International Honey Commission (IHC) and physicochemical parameters such as colour, water content, electrical conductivity, pH, free acidity, specific rotation, diastase activity and hydroxymethylfurfural (HMF) were determined in this study. The values obtained from rape honey samples were in the range: water content 15.60 – 18.40%, electrical conductivity 0.16 – 0.27 mS/cm, pH 3.75 – 4.15, free acidity 13.23 – 16.17 meq/kg. For coriander honey samples the following values were defined: water content 15.60 – 17.00%, electrical conductivity 0.37 – 0.58 mS/cm, pH 3.70 – 4.25, free acidity 14.70 – 19.60 meq/kg. The specific rotation in both honey types was negative. Colour, diastase activity and HMF were also discussed.

Key words: rape honey, coriander honey, physicochemical parameters, unifloral honeys.

Introduction

Honey is a natural product which has been used as a food since ancient times. It is an important and unique food product containing bioactive compounds. The main ingredients of honey are sugars (fructose and glucose). The amount of water should be up to 20% (*Council directive, 2001*). Honey also contains a wide variety of other substances in small concentrations such as organic and inorganic

acids, vitamins, enzymes, amino acids, mineral elements, flavonoides (*Golob and Plestenjak, 1999*). According to botanical origin, the main types of honey are blossom or nectar honey, obtained from the nectar of plants, and honeydew honey. Their characteristic properties are depending on the floral source of the nectar (*Persano Oddo et al.,2004*).

The determination of the botanical origin is part of the quality analysis of honey. Recently, there is an increasing commercial interest to produce unifloral honeys. Actually, many consumers prefer unifloral to multifloral honeys and appreciate the possibility to choose between different honey types. Unifloral honeys have also a higher commercial value for the producers than multifloral honeys. Thus, they can be considered as premium products. Moreover, the increasing interest in the therapeutic or technological uses of certain honey varieties may also contribute to the demand of a reliable determination of their botanical origin (*Ruoff et al., 2007*). In Bulgaria it is produced mainly multifloral honey and some types of unifloral (acacia, lime) honey and honeydew honey. The same honey types are commercially available on the market. Very scarce data is available regarding physicochemical parameters of Bulgarian rape honey (*Brassica spp.*) and coriander honey (*Coriandrum sativum L.*). Physicochemical parameters of the coriander honey have not been described in European unifloral honeys (*Persano Oddo and Piro, 2004*). Until now there has been no full research that determines the physicochemical parameters of Bulgarian coriander honey, except for *Atanassova et al. (2012)*, *Dinkov and Ivanov (2010)*, *Ivanov (1973)*. Though, these authors presented only few physicochemical parameters.

The goal of the present work was, first, to verify and compare some of the qualitative parameters such as water content, electrical conductivity, specific rotation, pH, free acidity, diastase activity and hydroxymethylfurfural (HMF) colour. Second, it aimed to increase the data for these unifloral honey types in Bulgaria.

Material and Methods

Seven rape honey samples and six coriander honey samples were analyzed. These samples were obtained from beekeepers. All samples were examined after being stored for one year at 25 ± 5 ^{o}C.

The following chemical parameters were determined according to the harmonized methods of the International Honey Commission (IHC) (*Bogdanov et al., 1997*) and the methods described in *BDS 3050-80*: colour, water content, electrical conductivity, pH, free acidity, hydroxymethylfurfural (HMF) after White, diastase activity according to *BDS 3050-80*. Honey samples were heated up to 40 °C to dissolve the crystals, and the colour was determined by Lovibond® Honey

ColorPod. The results were expressed in the Pfund scale (mm Pfund). Declared botanical origin of honey samples was verified by pollen analysis in the Central Laboratory for Veterinary Sanitary Expertise and Ecology, Sofia, Bulgaria. Analysis was performed by counting of pollen grains, according the method of Bulgarian regulation (*BDS 3050-80*). Statistical analysis was performed using MS Excel 2007. All results are presented as means ± standard deviation.

Results and Discussion

According to the obtained results, *Brassica spp.* pollen grains are in all analyzed samples represented as predominant pollen (>60% in total pollen). *Coriandrum sativum* pollen grains in all samples varied from 28% up to 59% giving the average of 48%. Afterwards, samples were subjected to the analysis of physicochemical parameters. In addition, *Dinkov and Ivanov (2010)* found in Coriander honey above 45% *Coriandrum sativum* pollen. This value is in agreement with our average pollen content.

The physicochemical parameters of honey are rather varying and primarily depend on the floral source. However, certain external factors also play a role such as seasonal and environmental factors. Tables 1 and 2 report the physicochemical parameters of Bulgarian rape and coriander honey.

Table 1. Physicochemical parameters of rape honey

Parameters	Mean±SD	Min	Max
Colour, mm Pfund	25±6	17	33
Water content, %	17.34±1.00	15.60	18.40
pH	3.91±0.16	3.75	4.15
Free acidity, meq/kg	14.98±1.16	13.23	16.17
Electrical conductivity, mS/cm	0.21±0.04	0.16	0.27
Specific rotation, $[\alpha]^{D}_{20}$	-16.57±1.99	-19.00	-12.50
Diastase activity, Gothe units	23.09±3.00	19.19	26.30
HMF, mg/kg	15.13±2.70	11.68	19.16

Colour is useful criterion for the classification of unifloral honeys (*Krell, 1996; Gonzales et al.,1999*). Visually, colour of rape honey samples is light and varies from 17 to 33 mm Pfund. According to *Szczesna et al. (2011)* the colour of Rape honey ranged from 8 to 59 mm Pfund. In comparison to rape honey, coriander honey samples have light amber colour (from 41 to 68 mm Pfund) (Table 2).

Water content is one of the parameters which provide information on the resistance of honey to spoilage by fermentation. The water content of honey depends on various factors, for example: the harvesting season, the degree of maturity reached in the hive, and environmental factors (*Acquarone et al., 2007; Feás et al., 2010*). The small variation observed in the water contents of these samples may be due to the similar practices for honey production applied by the beekeepers. The lower the water content, the lower the probability that honey ferments during storage. All samples have low water content, while electrical conductivity, pH value and acidity are inherent for blossom honey. As can be seen from Tables 1 and 2, water content, pH and free acidity values show good maturity and absence of undesirable fermentation in all the samples. The low pH of honey inhibits the presence and growth of microorganisms. This parameter is of great importance during the extraction and storage of honey as it influences its texture, stability and shelf life (*Terrab et al., 2004*). pH values for the two honey types are almost identical. The average values for pH in rape honey samples are fairly similar to those reported by *Popek (2002)*. *Atanassova et al. (2012)* found higher pH values for coriander honey. The same authors noticed that pH was slightly lower in rape honey than in our research. Free acidity of coriander honey samples are, however, slightly higher, 16.09±1.87 meq/kg compared to 14.98±1.16 meq/kg in rape honey.

Table 2. Physicochemical parameters of coriander honey

Parameters	Mean±SD	Min	Max
Colour, mm Pfund	58±10	41	68
Water content, %	16.27±0.59	15.60	17.00
pH	3.96±0.22	3.70	4.25
Free acidity, meq/kg	16.09±1.87	14.70	19.60
Electrical conductivity, mS/cm	0.44±0.08	0.37	0.58
Specific rotation, $[\alpha]^{D}_{20}$	-12.25±3.97	-18.50	-8.75
Diastase activity, Gothe units	15.02±1.46	12.43	16.50
HMF, mg/kg	17.38±1.41	15.87	19.92

Electrical conductivity values varied from 0.16 mS/cm to 0.27 mS/cm (Table 1) for the rape honey and from 0.37 to 0.58 mS/cm for the coriander honey (Table 2). The average values for the two honey types are similar to those reported by *Atanassova et al. (2012)*. The results for electrical conductivity in rape honey are concurrent with the results of *Szczesna et al. (2011)*.

Negative values of specific rotation are typical for nectar honey (*Bogdanov et al., 2004*). The values of the specific optical rotation for all samples were negative which is consistent with other results for unifloral honeys (*Persano Oddo et al., 1995*). Our values for specific rotation (see Table 2) for Coriander honey are considerably higher than values reported by *Dinkov and Ivanov (2010*).

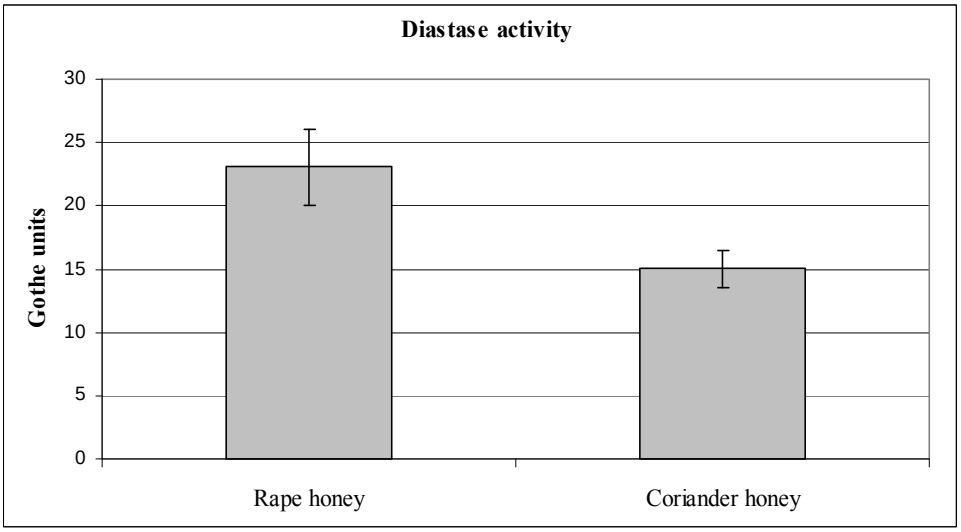

Fig.1. Diastase activity of rape and coriander honey

Storage has a remarkable influence on the increase in HMF content and the decrease in diastase number of the honeys collected. According to *Persano Oddo and Piro (2004)* the concentration of HMF (≤ 40 mg/kg) and the diastase activity values (≥ 8 in units of Schade) recorded are those of honeys of high quality. In honey, HMF is related to its quality and heat processing but opposite is not true – the latter two are not related to the honey type. Mean HMF contents in the rape and coriander honey are 15.13±2.70 and 17.38±1.41 mg/kg, respectively one year after the honey harvesting. After one year storage *Thrasyvoulou (1986)* found that the average HMF contents increased from 0.0 to 8.8 mg/kg. *Sancho et al. (1992)* found that the average amount of HMF increased from 4.7 to 13.1 mg/kg for the same storage period. Similar data is reported by *Yilmaz and Küfrevioglu (2001)*. After one year storage the average HMF content is 19.1 mg/kg. The rates of HMF in all honey samples in our study showed similarity to those mentioned above. Furthermore, HMF content for all samples (11.68 – 19.92 mg/kg, average 16.19 mg/kg) purports the fact that the samples were not fresh. But these samples are

representative and very often the honey production could not be sold at the same year of harvesting. However, it was found that HMF contents in all honey samples were lower than 40 mg/kg (Tables 1 and 2).

Rape honeys showed the highest average value of diastase activity (23.09 Gothe units). The diastase activity of Coriander honey ranged from 12.43 to 16.50 Gothe units. These results are comparable to the results reported by *Ivanov (1973)* for this honey type. As can be seen from Fig. 1 rape honey has higher diastase activity that coriander honey.

Conclusion

Considering physicochemical parameters, coriander honey and rape honey are similar to the same honey types produced in other countries. The physicochemical parameters such as water content, diastase activity and HMF showed values in agreement with the legal limits. The mean values of electrical conductivity, colour, free acidity for coriander honey are: 0.44±0.08 mS/cm, 58±10 mm Pfund and 16.09±1.87 meq/kg, respectively. For rape honey the following mean values were obtained: electrical conductivity 0.21±0.04 mS/cm, colour 25±6 mm Pfund, free acidity 14.98±1.16 meq/kg. These parameters related to the floral origin of honey. Taking into account all the results that we have reported in this paper, we suggest that physicochemical parameters of the coriander honey should be described in European unifloral honeys.

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Fizičko-hemijski parametri bugarskog meda od repice (*Brassica* spp.) i korijandera (*Coriandrum sativum l.*)

R. Balkanska, M. Ignatova

Rezime

Med se u suštini koristi kao hrana u celom svetu još od davnina. Med sadrži šećere, organske kiseline, enzime, aminokiseline, mineralne elemente, polen. Sastav meda varira u zavisnosti od izvora cvetnog meda.

Cilj ove studije je bio da se provere i uporede neki fizičko-hemijski parametri meda od repice (*Brassica* spp.) i korijandera (*Coriandrum sativum L.)* u uzorcima iz Bugarske i da se obogati baza podataka za ove tipove jednocvetnog meda. Sedam uzoraka meda od repice i šest uzoraka meda od korijandera su analizirani. Kriterijumi kvaliteta meda određeni su od strane Međunarodne komisiji za med (IHC) a fizičko-hemijski parametri, kao što su boja, sadržaj vode, elektro-provodljivost, pH, slobodna kiselosti, specifična rotacija, aktivnost dijastaze i hidrokimethilfurfural (HMF), su utvrđeni u ovoj studiji.

Vrednosti dobijene od uzoraka meda repice bili su u opsegu: sadržaj vode 15,60 - 18,40%, električna provodljivost 0,16 - 0,27 mS / cm, pH 3,75 - 4,15, slobodna kiselost 13.23 - 16.17 mEk / kg.

Za uzorke meda korijandera sledeće vrednosti su definisane: sadržaj vode 15.60 - 17,00%, električna provodljivost 0,37 - 0,58 mS / cm, pH 3,70 - 4,25, slobodna kiselost 14,70 - 19,60 mEk / kg. Specifična rotacija u obe vrste meda je bila negativna. Boja, aktivnost dijastaze i HMF su takođe razmatrani.

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FIRST MOLECULAR DETECTION OF SIX HONEYBEE VIRUSES IN SERBIAN APIARIES

T. Petrović[1], D. Vidanović[2], I. Stojanov[1], Diana Lupulović[1], G. Lazić[1], N. Plavša[3], S. Lazić[1]

1Scientific Veterinary Institute "Novi Sad", Rumanački put 20, 21000, Novi Sad, Republic of Serbia
2 Veterinary Specialized Institute Kraljevo, Žička 34, 34000, Kraljevo, Republic of Serbia
3Faculty of Agriculture, University of Novi Sad, Trg D. Obradovića 8, 21000, Novi Sad, Republic of Serbia
Corresponding author: tomy@niv.ns.ac.rs
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Abstract: The scientific interest in viral diseases of the honeybee (*Apis mellifera*) has been increasing considerably during the last decade. At least 18 different viruses have been detected in honeybees so far, but six of them are considered to be able to cause severe disease. Clinical signs of disease and suspicions on the bee viruses presence in Serbian apiaries exist for many years, but with no laboratory confirmation data. This research describes the detection of six honeybee viruses: acute bee paralysis virus (ABPV), black queen cell virus (BQCV), chronic bee paralysis virus (CBPV), deformed wing virus (DWV), Kashmir bee virus (KBV), and sacbrood virus (SBV) in 30 bee samples originated from 15 apiaries at 13 locations in Southern Backa (7) and Srem district (6) in Serbia. Using one-step reverse transcription-PCR (RT-PCR), bee virus presence was detected in all examined samples (100%). The most prevalent virus was BQCV, present in all 30 (100%) samples, followed by SBV, DWV, CBPV and ABPV (detected in 21 (70%), 20 (67%), 18 (60%) and 17 (57%) samples respectively), while KBV was not detected in any sample. In most samples (96.67%), more than one virus was identified. In most of the tested samples (96.67%), more than one virus was identified. Only 1 analyzed sample (3.4%) were infected with one virus, 2 (6.7%) with two viruses, 14 (46.7%) with three viruses, 6 (20%) with four viruses and 7 (23.3%) samples contained five viruses simultaneously. The results of this study indicate a high prevalence of 5 out of 6 examined bee viruses in Serbian apiaries, and the great burden of surveyed apiaries caused by multiple viral infections. At the same time, these results require further and more extensive research.

Key words: honeybee, bee viruses, diagnosis, RT-PCR, Serbia

Introduction

The honey bee *Apis mellifera* is an important beneficial insect that play an essential role in the ecology of natural environments and in agricultural production through pollination. Also, honeybees play an equally crucial role in conserving the biodiversity in many natural ecosystems. The value of honeybee pollination for agriculture has been estimated at more than $14.6 billion in the United States (*Morse and Calderone, 2000*). According to FAO and the European Union, the value of pollination is 20–30 times higher than the value of honey production (*Antúnez et al., 2012*).

However, the honeybees' health is often compromised by different pathogens, such as the mites *Varroa destructor* and *Acarapis woodi*, the microsporidia *Nosema ceranae* and *Nosema apis*, the bacteria *Paenibacillus larvae* and *Melissococcus plutonius* and different viruses (*Antúnez et al., 2012*). Viruses are probably the least understood part of honeybee pathology mainly because of the lack of information of the objective data about viral disease outbreaks (*Toplak et al., 2012*). So far, honey bees have been reported to be the host to at least 18 viruses (*Allen and Ball, 1996*). Most of them cause unapparent infections without clinical signs but in certain cases may cause serious or lethal diseases (*Allen and Ball, 1996; Antúnez et al., 2012*). Of those viruses infecting honeybees, seven viruses are considered to be able to cause severe disease. Acute Bee Paralysis Virus (ABPV), Chronic Bee Paralysis Virus (CBPV), Deformed Wing Virus (DWV), Black Queen Cell Virus (BQCV), Sacbrood Virus (SBV), Kashmir Bee Virus (KBV), and Israeli Acute Paralysis Virus (IAPV) may be considered among the most important viruses that affect honeybees (*Chen and Siede, 2007; de Miranda et al., 2010; de Miranda and Genersch, 2010; Antúnez et al., 2012; Toplak et al., 2012*).

In nature, BQCV, DWV, KBV, and SBV infect larvae and pupae as well as adult bees, while ABPV affects only adult bees (*Chen et al., 2004*). BQCV was first detected in brown and black coloured queen larvae and prepupae but it also affects larvae and pupae of worker bees without causing signs (Antúnez et al., 2012). Infection with DWV is typically associated with *V. destructor* presence and results in deformed wings of honeybees. It is one of the most studied viruses that affect honeybees due to its relation with colony losses induced by honeybee's mite *V. destructor*. SBV affects larvae of honeybees and causes sacbrood disease. Affected larvae change from pearly white to gray and finally black. When affected larvae are carefully removed from their cells, they appear to be a sac filled with water. CBPV induces abnormal trembling of the wings and body of honeybees. The wings are partially spread or dislocated. In some cases the crawling bees can be in large numbers representing the clinically evident disease. ABPV, KBV and

IAPV are the complex of related viruses. At low levels, infected colonies do not show clinical signs, but the high level of those viruses induces high mortality rates (*Antúnez et al., 2012*). These viruses have been associated with honeybee colony losses, especially when colonies are co-infected with *V. destructor* (*de Miranda et al., 2010; Antúnez et al., 2012*). Diagnosis of bee virus infections is difficult because honey bee viruses usually persist as non-apparent infections and cause no clinical signs of disease, and because the fact that bee colonies can be attacked by more than one virus in the same time, as well as including other pathogens. Multiple viral infections have been reported in bees by a number of authors (*Anderson and Gibbs, 1988; Chen et al., 2004; Toplak et al., 2012*).

Molecular methods as reverse transcription polymerase chain reaction (RT-PCR) enable revolution in the diagnosis of virus infections and offers highly specific and sensitive method for virus detection. Several studies used a RT-PCR assay to detect and identify virus infections in honey bee colonies (*Stoltz et al., 1995; Bakonyi et al., 2002; Benjeddou et al., 2001; Chen et al., 2004a; Cizelj and Gregorič, 2004; Blanchard et al., 2007; Antúnez et al., 2012; Toplak et al., 2012*). RT-PCR methods that were used in all these studies confirmed that are highly specific and sensitive for detection of viruses in honeybee samples and suitable for surveys of viruses presence in bee colonies in regional and national levels. In the last few years many researchers using RT-PCR methodology reported individual and more often multiple virus detection in bees colonies in Europe and worldwide (*Berenyi et al., 2006; Baker et al., 2008; Forgách et al., 2008; Antúnez et al., 2012; Toplak et al., 2012*).

The first detection of viruses in Serbian apiaries has been done by Kulinčević and collaborators even 27 years ago (*Kulinčević et al., 1990*). In that study, samples of adult honey bees have been collected from 2 apiaries near Belgrade in autumn 1986 and early spring 1987 and tested on virus presence by electron microscope and immunodiffusion test (with different bee virus antisera). The results of the study confirmed the presence of 4 bee viruses, namely: ABPV, Cloudy Wing Virus (CWV), J strain of Egypt bee virus (now called DWV) and BQCV in Serbian apiaries.

The aim of our studies was to evaluate the present situation on the presence and distribution of different honeybee viruses in Serbian aperies. In this paper we described the results of the first survey of bee viruses in Serbia based on the molecular diagnostic methods, with the prevalence of six RNA viruses in samples collected in the apiaries in two Counties in Vojvodina province - northern part of Serbia from 2011 to 2013. The aim of this survey was also to establish the routine molecular laboratory methodology for specific detection of honey bee viruses.

Material and Methods

Samples

Thirty samples of worker honeybees (*Apis mellifera*) were collected from 15 different apiaries from 13 locations in Southern Backa (7) and Srem County (6) of Vojvodina province in Serbia. Bee samples were collected from June 2011 to May 2013 and were of different health status from apparently clinically normal colonies to ill or dead bees associated with abnormal mortally or sudden colony losses. Each sample consisted of a pool of about 100 bees of the same beekeeper and in a few cases more than one sample was collected from the same apiary. For the analysis 10 bees were randomly selected from each obtained bee sample and homogenized by mortar and pestle in 5 mL of sterile PBS. After homogenization, the samples suspensions were centrifuged for 10 min at 3000 rpm. The supernatant was collected and immediately used for RNA extraction.

Nucleic acid extraction and RT-PCR analysis

For RNA extraction 140 µl supernatants of bee homogenates was used. Total RNA was extracted using QIAamp viral RNA mini kit (Qiagen, Germany) according to manufacturer's instructions. Each RNA sample was individually tested for the presence of genomes nucleic acids of six viruses. Part of viral genome of ABPV, BQCV, CBPC, DWV, KBV and SBV was amplified by RT-PCR using specific primer pair (Table 1) and One-Step RT-PCR kit (Qiagen, Germany) reagents according to the manufacturer's instructions. Briefly, the reaction was performed in a total volume of 25 µl as follow: 12 µl of nuclease free water, 5 µl of 5 x PCR buffer, 1 µl of dNTP mix (containing 10 mM of each dNTP), 1 µl of 20 µM solution of each primer, 1 µl of one step RT-PCR enzyme mix and 5 µl of RNA template. Virus positive controls and no template controls were also performed for each virus and each reaction. The details about the primers used in our study are shown in Table 1. The PCR program included reverse transcription stage at 50 □C for 30 min, followed by an initial PCR activation step at 95 □C for 15 min. This was followed by 40 cycles of 94 □C for 30 sec, 54 □C for 30 sec and 72 □C for 1 min and final extension step at 72 □C for 10 min. The annealing temperature for DWV and CBPV was 60 and 55 □C, respectively. The obtained PCR products were visualized in 1.5 % agarose gel with 0,5µg/ml ethidium bromide solution and under UV light. The size of each PCR product was compared to the 100-bp DNA ladder (Fermentas, Germany).

Table 1. Details of oligonucleotide primers used in this study

Primers (5'-3')	Position in genome	Product (bp)	Reference
Acute bee paralysis virus			
ABPV-1 (cat att ggc gag cca cta tg)	8114 - 8512	398	Bakonyi et al., 2002
ABPV-2 (cca ctt cca cac aac tat cg)	(capsid protein)		
Black queen cell virus			Benjeddou et al., 2001
BQCV-F (tgg tca gct ccc act acc tta aac)	7850 - 8550	700	
BQCV-R (gca aca aga aga aac gta aac cac)	(structure polyprotein)		
Chronic bee paraliysis virus			Blanchard et al., 2007
CBPV1-1 (tca gac acc gaa tct gat tat tg)	147-716	570	
CBPV1-2 (act act aga aac tcg tcg ctt cg)	(RNA polymerase)		
Deformed wing virus			Cizelj and Gregorič, 2004
DWV F (agg cga cat ggg aac agg)	1312-1815	504	
DWV R (caa ctt cac cct cgc cat ca)	(capsid protein)		
Kashmir bee virus			
KBV 1 F (gat gaa cgt cga cct att ga)	5406 - 5820	414	Stoltz et al., 1995
KBV 1 R (tgt ggg ttg gct atg agt ca)	(RNA polymerase)		
Sacbrood bee virus			
SBV-F (gct gag gta gga tct ttg cgt)	(4957-5781)	824	Chen et al., 2004/a
SBV-R (tca tca tct tca cca tcc ga)	(structure polyprotein)		

Results and Discussion

The scientific interest in viral diseases of the honeybee (*Apis mellifera*) has been increasing considerably during the last decade due to the high honeybee colonies losses observed worldwide. In this paper we describe, by our knowledge the first molecular detection of six honeybee viruses in the honeybee samples collected from apiaries in Serbia.

Honeybee virus presence was detected by one-step RT-PCR in all 30 (100%) examined bee samples collected from 15 different apiaries from 13 locations in Southern Backa (7) and Srem County (6) in Serbia between June 2011 and May 2013. The most prevalent virus found was BQCV and it was present in all 30 (100%) samples, followed by SBV detected in 21 (70%), DWV in 20 (67%), CBPV in 18 (60%) and ABPV detected in 17 (57%) samples respectively. Presence of KBV was not detected in any sample (Table 2). During the RT-PCR testing all the negative controls remains negative and the positive controls produce clear visible PCR bend of expected size.

Among 20 analyzed honeybee samples from 9 apiaries at 7 locations in South Bačka County all 20 (100%) samples was positive on the presence of

BQCV, 17 (85%) on the presence of CBPV, 16 (80%) on the presence of SBV, 11 (55%) on the presence of DWV and 10 (50%) samples on the presence of ABPV. The results of virus presence in 10 bee samples from 6 apiaries and 6 locations on the territory of Srem County were slightly different. The most prevalent viruses found were BQCV in 100% (10/10) and DWV in 90% (9/10) tested samples, followed by ABPV found in 70% (7/10), SBV found in 40% (4/10) and CBPV found in only 1 out of 10 (10%) examined samples (Table 2). If we exclude BQCV, the most prevalent bee virus found in Southern Bačka County was CBPV and in Srem County was DWV. This difference could not be seen in clinical manifestation of the disease on the field. Maybe one of the reasons is the relative small number of examined samples or not enough detailed anamnesis data obtained from the field.

The obtained results also indicate a high burden of surveyed apiaries caused by multiple viral infections. In most of the tested samples (96.67%), more than one virus was identified. Only 1 analyzed sample (3.4%) were infected with one virus (BQCV), 2 (6.7%) with two viruses, 14 (46.7%) with three viruses, 6 (20%) with four viruses while 7 (23.3%) samples contained five viruses simultaneously (Table 3). Almost half of the analyzed samples had 3, and more than 40% of the analyzed samples had 4 or 5 simultaneous viral infections. The severities of clinical manifestation with high bee losses were associated with higher number of viruses detected in the samples. These results also demonstrated that mixed virus infections in honeybees are quite widespread in Serbia as we detected mixed infections of ABPV, CBPV, BQCV, DWV, KBV, and SBV in adult worker bees, and are in correlation with the data reported from other countries (*Chen et al., 2004; Tentcheva et al., 2004; Berenyi et al., 2006; Antúnez et al., 2012; Toplak et al., 2012)*.

In the field, honey bee colonies can suffer from multiple virus infections without showing obvious pathological symptoms, thereby confounding diagnoses. A rapid and accurate diagnosis for virus infection, therefore, is necessary for honey bee disease surveillance and control programs. The PCR method has become the standard, reliable, rapid, specific and accurate method for detecting many pathogens. The RT-PCR methodologies that we used in this study were proved to be rapid, highly specific and sensitive for detection of determined bee viruses, and for the surveillance studies of viruses presence in honeybee colonies.

Table 2. Honeybee viruses (ABPV, CBPV, SBV, DWV, BQCV and KBV) detected in examined 30 honeybee samples from 2 Counties of Vojvodina province in Serbia

	Sampl No	Apiary No	Location	Year	Presence of bee viruses by RT-PCR					
					ABPV	CBPV	SBV	DWV	BQCV	KBV
South Bačka County	1	1	Bačka Palanka	2012	+	+	+	+	+	-
	2				+	+	+	+	+	-
	3				-	+	+	+	+	-
	4				-	+	+	+	+	-
	5	2	Bačka Palanka	2011	-	+	+	-	+	-
	6	3	Mladenovo	2013	+	+	+	+	+	-
	7				+	+	+	+	+	-
	8				+	+	+	+	+	-
	9	4	Bač	2011	-	+	+	-	+	-
	10	5	Bač	2011	-	-	+	-	+	-
	11	6	Srbobran	2012	+	+	-	-	+	-
	12				+	+	-	-	+	-
	13				+	+	-	-	+	-
	14	7	Bečej	2013	-	+	+	-	+	-
	15				-	-	-	-	+	-
	16				-	+	+	-	+	-
	17	8	Kisač	2013	-	+	+	+	+	-
	18				-	+	+	+	+	-
	19	9	Kovilj	2013	+	+	+	+	+	-
	20				+	-	+	+	+	-
Srem County	21	10	Susek	2013	+	+	+	+	+	-
	22				+	-	+	+	+	-
	23	11	Popovica	2011	-	-	+	+	+	-
	24	12	Iriški venac	2013	+	-	-	+	+	-
	25				+	-	-	+	+	-
	26	13	S. Mitrovica	2013	-	-	+	-	+	-
	27	14	Jarak	2013	+	-	-	+	+	-
	28				+	-	-	+	+	-
	29				+	-	-	+	+	-
	30	15	Petrovaradin	2013	-	-	+	+	+	-
TOTAL					**17 57%**	**18 60%**	**21 70%**	**20 67%**	**30 100%**	**0 %**

Honeybee viral prevalence found in Serbia was similar or even higher comparing with the situation observed in other countries.

BQCV was found in all analyzed samples (100%) in our study. This virus was also high prevalent in the first study on honey bee virus presence in Serbia were it was detected in 50% of samples, and all BQCV positive colonies were collapsed during winter time (*Kulinčević et al., 1990*). This virus infection has been reported to have a variable prevalence in different countries, with 10, 54, 83, 86, 30 and 90% detection rates in Spain, Hungary, Slovenia, France, Austria and Uruguay, respectively (*Antúnez et al., 2012; Berenyi et al., 2006; Forgach et al., 2008; Toplak et al., 2012; Tentcheva et al., 2004; Antunez et al., 2006*). The reason of this high BQCV prevalence in Serbian apiaries is not quite clear. It is previously observed that infections of bees with BQCV were often associated with the infestation of the protozoan bee parasite *Nosema apis* (Bailey et al., 1983). We didn't tested our bee samples on *Nosema* presence, but it is known that this parasite is highly prevalent in Serbian apiaries, so maybe this could be one of the explanation of the found high BQCV prevalence. Although adult bees are often infected with BQCV, they normally do not exhibit the disease symptoms.

Table 3. Detection of multiple virus presences in analyzed honeybee samples

No of detected viruses	Detected viruses	No of samples	% of samples
0 virus	/	0	0%
1 virus	BQCV	1	3.4%
2 viruses	SBV and BQCV	2	6.7%
3 viruses*	BQCV, DWV, CBPV, ABPV or SBV	14	46.7%
4 viruses	SBV, DWV, BQCV and CBPV or ABPV	6	20%
5 viruses	ABPV, CBPV, SBV, DWV and BQCV	7	23.3%
6 viruses	/	0	0%
Total		**30**	**100%**

* Combination of three out of 5 listed viruses

The second high prevalent bee virus in our study was SBV found in 70% of tested samples. This result is very similar to those obtained in France, Austria and Uruguay were SBV was found in 86, 48 and 100% samples, respectively (*Tentcheva et al., 2004; Berenyi et al., 2006; Antunez et al., 2006*). However, these values were much lower in Spain (1.1%), England (1.4%), Hungary (2%) and Slovenia (8.3%) (*Antúnez et al., 2012; Baker and Schroeder, 2008; Forgach et al., 2008; Toplak et al., 2012*). Sacbrood virus appears mainly in spring, when the brood season begins and large numbers of infected young adults are present

(*Berenyi et al., 2006*). Bee samples in our study were mainly collected during the spring time, so except the fact that all tested viruses were found in high prevalence in our study, this could be one of the explanations of detected high SBV prevalence.

ABPV, CBPV and DWV are virus infections usually connected with clinical manifestation of disease among adult bees. Mortality and paralysis were often evident in the apiaries with ABPV and/or CBPV infections and both viruses were detected in clinically affected apiaries where flightless bees, trembling and crawling of bees were observed (*Toplak et al., 2012*). In colonies weakened by the Varroa mite, cold weather, nutrition problems, or intoxication, ABPV is considered to be the main causative agent of depopulation and collapse of the colonies (*Nordstom et al., 1999*). During CBPV infection in some cases up to 30% of worker bees could be affected and die. DWV is also among viruses connected with the sudden loss and collapse of the bee colonies especially in colonies highly infested with the Varroa mite. This virus is considered to be the most widespread bee virus worldwide. Its presence is often associated with *V. destructor* infestation, and the role of the mite in transmission of the virus has already been experimentally demonstrated (Bowen-Walker et al., 1999). Afore mentioned viruses were found in 67% (DWV), 60 (CBPV) and 57% (ABPV) examined bee samples in Serbia. The prevalence of DWV in Serbian apiaries is similar with the findings in Hungary (72%) and Slovenia (70%) (*Forgach et al., 2008; Toplak et al., 2012*) and slightly lower than 97%, 91%, 97% and 100% found in France, Austria, England and Uruguay (*Tentcheva et al., 2004; Berenyi et al., 2006; Baker and Schroeder, 2008; Antunez et al., 2006*). ABPV and DWV were also detected in Serbian apiaries 27 years ago, in 1986 (*Kulinčević et al., 1990*). The obtained prevalence of CBPV in the present study (60%) is much higher than those reported in other European and surrounding countries. CBPV was rarely detected in the French (28%), Slovenian (18%) and Austrian (9%) surveys (*Berenyi et al., 2006; Toplak et al., 2012; Tentcheva et al., 2004*). However, obtained prevalence (57%) of ABPV in Serbian apiaries is similar to those reported in Slovenia (40%), Hungary (37%), France (58%) and Austria (68%) (*Toplak et al., 2012; Forgach et al., 2008; Tentcheva et al., 2004; Berenyi et al., 2006*).

KBV was not detected in any of 30 examined honeybee samples in the present study. These results are in accordance with the data reported from Hungary, Austria or Uruguay where KBV was not detected (*Antunez et al., 2006; Berenyi et al., 2006; Forgach et al., 2008*). This virus is widespread in the United States and Australia, but it has rarely been detected in Europe (*Allen and Ball, 1996*). So far, the obtained detection rate was very low in Spain (0.3% in 2006 and 1.0% in 2007) (*Antúnez et al., 2012*), and in Slovenia 1.7% (1/60) (*Toplak et al., 2012*), while in France 17% of KBV positive samples were detected (*Tentcheva et al., 2004*).

The high prevalence of tested bee viruses found in Serbian apiaries could be the result of the high densities of bee colonies in geographic regions from where bee samples were collected. The intensive trade and transport of bee colonies, queens, hives, or equipment should also be taken into consideration as possible important factors for transmission and spread of these viruses between apiaries. The obtained results are just the first preliminary findings after the long period of laboratory undiagnosed or under diagnosed situations after the first detection of virus presence in Serbian apiaries that was conducted 27 years ago (*Kulinčević et al., 1990*) and more extensive studies are needed for better understanding of epidemiological situation.

Conclusion

To summarize, we have looked into the occurrence of the six most important honeybee viruses in Serbian bee colonies and identified high prevalence of 5 of them, namely BQCV (100%), SBV (70%), DWV (67%), CBPV (60%) and ABPV (57%). Presence of KBV was not detected on any of tested samples. The obtained results also indicate a high burden of surveyed apiaries caused by multiple viral infections. In approximately 90% of analyzed bee samples 3 or more virus multiple infections was detected. This high prevalence of tested bee viruses found in Serbian apiaries may be partly explained by the high densities of bee colonies in geographic regions from where bee samples were collected. The intensive and uncontrolled trade and transport of bee colonies, queens, or equipment could also be one of the reasons for transmission and spread of these viruses between apiaries. The RT-PCR techniques described in this paper proved to be appropriate methods for rapid, specific and sensitive detection of tested bee viruses, and for the surveillance studies of viruses presence in honeybee colonies. The obtained results are just the preliminary findings and more extensive studies are needed for better understanding of epidemiological situation.

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Prva molekularna detekcija šest virusa pčela u pčelinjacima Srbije

T. Petrović, D. Vidanović, I. Stojanov, Diana Lupulović, G. Lazić, N. Plavša, S. Lazić

Rezime

Naučni interes za virusne bolesti pčela (*Apis mellifera* L.) se značajno povećao u poslednjih deset godina. Do sada je najmanje 18 različitih virusa utvrđeno kod pčela ali se za njih 6 smatra da mogu uzrokovati klinički značajno oboljenje. Već duži niz godina postoje klinički znaci bolesti i sumnja na prisustvo virusnih infekcija u srpskim pčelinjacima ali bez laboratorijskih potvrdnih nalaza. Ovo istraživanje opisuje detekciju 6 virusa pčela: virus akutne paralize pčela (ABPV), virus crnog matičnjaka (BQCV), virus hronične paralize pčela (CBPV), virus deformisanih krila (DWV), Kašmirski virus pčela (KBV) i virus mešinastog legla (SBV) u 30 uzoraka pčela iz 15 pčelinjaka sa 13 lokacija u Južnobačkom (7) i Sremskom (6) okrugu u Srbiji. Upotrebom „one-step reverse transcription-PCR" (RT-PCR) tehnike prisustvo virusa je utvrđeno u svim ispitivanim uzorcima pčela (100%). Najveća prevalenca je utvrđena za BQCV koji je bio prisutan u svim ispitivanim uzorcima (100%), a zatim za viruse SBV, DWV, CBPV i ABPV (detektovanih u 21 (70%), 20 (67%), 18 (60%) i 17 (57%) uzoraka), dok KBV nije bio detektovan niti u jednom ispitanom uzorku. U većini uzoraka (96,67%) utvrđeno je prisustvo više od jednog virusa. Samo je za 1 (3,4%) uzorak utvrđeno da je inficiran jednim virusom, 2 (6.7%) su bila inficirana sa dva virusa, 14 (46.7%) sa tri virusa, 6 (20%) sa četiri virusa i 7 (23.3%) uzoraka su bila inficirana sa 5 virusa istovremeno. Rezultati ovih ispitivanja ukazuju na izuzetno visoku prevalencu 5 of 6 ispitivanih virusa pčela, kao i na veliku opterećenost ispitanih pčelinjaka višestrukim virusnim infekcijama u Srbiji. Istovremeno, dobijeni rezultati zahtevaju dalja i opsežnija istraživanja.

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GROWTH AND DEVELOPMENT OF SKELETAL MUSCLE IN CONNECTION WITH THE EXPRESSION OF THE MYOSTATIN GENE (MSTN)*

G. Nakev[1], P. Stamberov[2], I, Dimitrova[3], N. Stancheva[1], S. Georgieva[4], D. Hristova[4], G. Angelov[2], T. Mehmedov[2], K. Genova[2], A. Teneva[3]

[1] Agricultural Institute – Shumen, Bulgaria
[2] Faculty of Veterinary Medicine, University of Forestry, Sofia, Bulgaria
[3]Agronomy Faculty, University of Forestry, Sofia, Bulgaria
[4]Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria
Corresponding author: jivko_nakev@abv.bg
Review paper

Abstract: One approach of genetic selection is using genetic markers for the identification of breeding traits with economic importance. A gene which is already used as a marker gene is MSTN. It is responsible for the synthesis of the myostatin protein that regulates muscle growth. Mutations leading to loss of function (loss of-functional mutations) in the myostatin gene (MSTN) are associated with increased skeletal muscle mass ("doublemuscling"), which is connected with poor meat quality characteristics. In some cases it was found abnormal adipogenesis consisting in a reduced liptin secretion, changes in the structure of ligament, reproductive problems.

Key words: MSTN, genetic markers, skeletal muscle development.

Introduction

Improving the quantitative and qualitative characteristics of the carcass is one of the main tasks of modern genetics and selection. With the increase in the share of intellectual work, the requirements of consumers turned to search for lean meat with low fat content. Modern science has different methods to meet the market requirements. Nowadays the identification of breeding traits of economic importance was performed using marker genes. This review considered a key candidate genes myostatin (MSTN), related to improving the quality of the carcass in farm animals.

Prenatal and postnatal development of skeletal muscle

Efficient meat production can be achieved with animals which are characterized by rapid growth and a minimum fat deposition. During embryonic muscle growth raising skeletal muscle mass was due to the increased number of muscle fibers (hyperplasia), and after birth - are the result of an increase in their size (hypertrophy) (*Wegner et al., 2000*). Some researchers have suggested that may have an increase in the number of muscle fibers immediately after birth (*Rehfeldt et al., 2000*) and it is most likely as a result of maturation and extending of muscle fibers during the postnatal period of muscle growth (*Ontell,Kozeka,1984*). *Fahey et al.* (*2005*) believes that the ability to manipulate the amount of muscle fibers in the fetus will have significant implications for post-embryonic growth of the animal. According to *Larzul et al.* (*1997*) growth potential of the muscles is determined by the number of prenatally formed muscle fibers and the speed of postnatal hypertrophy.

Growth and development of skeletal muscles in some farm animals in conjunction with the expression of myostatin gene.

Improving meat qualities of farm animals through genetic selection requires methods of scaling of muscle growth, which retain their wealth and not adversely affect product quality. One approach of the genetic selection is application of genetic markers for the identification of breeding traits of economic importance.

A gene which is already used as a marker gene is myostatin gene (MSTN). It is responsible for the synthesis of the myostatin protein that regulates muscle growth. It was discovered by *McPherron et al.*(*1997*) in a study performed on mice. The researchers have found that mice lacking the gene MSTN (Mstn/-), were 30% heavier than normal mice, while obesity is entirely made up of muscles. Myostatin (also known as growth and differentiation factor 8, GDF8) is a transforming growth factor beta protein that inhibits the skeletal muscle development and may regulate adipogenesis. This protein is produced primarily in the muscles, circulates in the blood and acts on muscle tissue, by binding to a receptor called the activin type 2 (*Lee,McPherron,2001*). According *Hickford et al.* (*2009*) GDF8 is a member of super-family covering a large number of factors affecting growth and differentiation of tissues in embryonic and post-embryonic development.

Mutations that lead to a loss of function (lossof-function mutation) in a myostatin gene are associated with an increase in skeletal muscle mass (doublemuscling) in mice (*McPherron et al., 1997;Mendias et al., 2008*), sheep (*Clop et al.,2006;Kijas et al.,2007;Han,2012*), cattle (*Grobet et al., 1997;*

Marchitelli et al., 2003; Esmailizadeh et al., 2008; Grisolia et al., 2009; Haynes et al., 2013), pigs (*Stinckens et al., 2005; Stinckens et al., 2008*) and chicken (*Baron et al., 2002*).

In cattle, a phenomenon, a "doublemuscling", (muscle hypertrophy, mh phenotype), is the subject of numerous studies (*Arthur P.,1995; Dunner et al.,1997;Grobet et al.,1997; McPherron, Lee, 1997; Smith et al., 1997*). Morphological "doublemuscling", is characterized by 20-25% increase in muscle, which shows the significant influence of MSTN on the amount of muscle tissue in the body (*Grobet et al., 1997; McPherron and Lee,1997*), mainly due to hyperplasia of muscle fibers (*Wegner et al., 2000*). Using *mh* phenotype in cattle production provides carcasses with a high proportion of lean meat (*Di Stasio,Rolando, 2005*) with reduced fat content (*Shahin,Berg,1985*). According to *Wiener et al.* (*2002*) MSTN in cattle has pleiotropic effect. It is located on chromosome 2 (2q14-q15) (*Grobet et al., 2003*). *Bellinge et al.* (*2005*) examined myostatin and its implications for breeding and they recognized that from nine known mutations in MSTN, six of them are responsible for the phenotypic expression of the muscle hypertrophy. The phenotype of this kind has a higher incidence in some cattle breeds, as Belgian Blue and Peidmontese, and is often accompanied with reproductive problems, which according to the same authors can be overcome by genetically controlled breeding programs. Unfavorable pleiotropic effects were also reported by other authors - increased levels of dystocia (*Short et al., 2002*), reduced fertility, calving difficulty and high mortality rates (*Wiener et al., 2002; Casas et al., 2004*).

Mh phenotype occurs at different frequencies in different cattle breeds, which is probably due to differences in the selection pressure, depending on market requirements (*Dunner et al., 2003*). The presence of myostatin g+ 6723G> A mutation in sheep leads to increased muscle mass and reduce fat in the body (*Laville et al.,2004;Clop et al.,2006; Kijas et al.,2007;Johnson et al., 2009*). A genetic survey of 326 animals in Australia revealed that SNP g+ 6723G>A mutation is near fixation within breed Texels and present in additional breeds including White Suffolk, Poll Dorset and Lincoln (*Kijas et al., 2007*). According to the same authors loss the function of allele (g+ 6723A) is associated with increased amount of muscle and reduce intramuscular fat in the body, which has a negative impact on meat quality. *Haynes et al.* (*2012*) studied the relationship between the amount received nutrients and qualitative and quantitative carcass characteristics of lambs with myostatin g+ 6723G> A mutation. The experiment shows that the low food supply in heterozygous lambs may adversely affect growth and muscle characteristics. According to these authors the study is of great importance because previous studies evaluating the effects of myostatin g+ 6723G> A mutation were

conducted in pasture breeding without control of feed intake by lambs (*Kijas et al., 2007; Jonhnson et al., 2009; Masri et al., 2011*).

In a critical analysis of identified to date scientific evidence for genetic variation in loci responsible for the formation of muscle in sheep *Tellam et al., (2012)* point out that the accumulation of new knowledge in this area could contribute to the development of biochemical and immunological strategies aimed at increasing the amount of muscle in the body.

In the pig-breeding Pietrain breed is characterized by phenotype similar to the "doublemuscling" in cattle. It is assumed that the genetic variants of the myostatin gene affect muscle growth (*Guimaraes et al.,2007;Stinckens et al., 2008*), but the mechanism by which MSTN affect muscle growth in pigs has not been elucidated completely, indicating the need for further research.

Conclusion

At the present stage intensification of meat production is inconceivable without the methods of genetic selection. The review on the issues of growth and development of skeletal muscle in connection with the expression of the Myostatin gene shows that science provides answers to many questions, but still remain substantial ambiguities in this area. This demonstrates the need to continue and deepen this kind of research whose ultimate goal is to meet the needs of consumers with high quality meat.

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Uticaj ekspresije miostatin gena (MSTN) na rast i razvoj skeletnih mišića

G. Nakev, P. Stamberov, I, Dimitrova, N. Stancheva, S. Georgieva, D. Hristova, G. Angelov, T. Mehmedov, K. Genova, A. Teneva

Rezime

Jedan od pristupa genetskoj selekciji je korišćenje genetskih markera za utvrđivanje priplodnih osobina od ekonomskog značaja. Gen koji se već koristi kao marker gen je MSTN. On je odgovoran za sintezu proteina miostatina koji reguliše rast mišića. Mutacije koje dovode do gubitka funkcije miostatin gena (MSTN) povezane su sa povećanjem mišićne mase (udvostručena mišićavost), što je povezano sa lošim karakteristikama kvaliteta mesa. U nekim slučajevima je utvrđena nenormalna adipogeneza koja se sastoji u smanjenom lučenju liptina, promenama u strukturi ligamenata i pojavi reproduktivnih problema.

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POSTER SECTION II

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FACTORS OF IMPORTANCE FOR SUCCESSFUL ELECTRICAL STUNNING OF PIGS

M. Dokmanović, M. Z. Baltić, N. Karabasil, N. Glamoclija, S. Pantić, R. Marković, M. Todorović[1]

[1]Faculty of Veterinary Medicine, Bulevar Oslobodjenja 18, 11 000 Belgrade, Serbia
Corresponding author: marijadok@gmail.com
Original scientific paper

Abstract: There are regulations in European Union, as well as in our country, which require that animals during slaughter must be unconscious, must not feel a pain and in this state must remain until complete loss of brain function due to exsanguination. Electrical stunning effectiveness in pigs can be assessed through two critical points, and these are the percentage of animals that are effectively stunned at the first attempt and the percentage of animals that remain insensitive during slaughter procedure. In order to properly perform stunning procedure workers have to be careful, trained and competent. In addition, the equipment used for stunning should be in such a way designed and constructed, as well as regularly cleaned and calibrated to ensure optimal current flow and effective stunning of pigs. Proper stunning is important both for high standard of animal welfare and meat quality, and therefore it is important to perform stunning procedure in accordance with recommendations.

Key words: pig, electrical stunning, welfare, staff, equipment

Introduction

In order to maintain a high standard of animal welfare during slaughter, Regulations of the EC require that animals must be unconscious, must not feel a pain and in this state must remain until complete loss of brain function due to exsanguination. The same requirements are set in our country where according to Regulation (*Anon, 2010*), animals must be stunned before slaughter in a manner that causes instantaneous loss of consciousness. Shackling and hoisting of stunned pigs do not cause anxiety, pain, suffering and stress (*Anon, 2004*). In addition, stress one minute prior to slaughter contributed to lower pH and higher temperature of meat 45 minutes after slaughter, as well as to a lower water holding capacity indicating low meat quality (*van der Wal, 1997*). In order to enhance animal welfare, it is necessary to monitor and assess stunning effectiveness using objective

measures (*Grandin, 2001; Grandin, 2010a; Grandin, 2010b*). Two critical points should be monitored during electrical stunning of pigs to assess animal welfare and success of stunning, and it is the percentage of animals that are effectively stunned at the first attempt and the percentage of animals that remain insensitive during slaughter procedure (*Grandin, 2010a*).

Effective stunning at the first attempt

Pigs are usually stunned using current or gas. With current pigs can be only stunned if electrodes are placed on the head, or stunned and killed, if current first passes through the head, and then through the heart (*Grandin, 2010a*). When assessing the effectiveness of head-only electrical stunning, it is necessary to check whether electrodes are placed in the correct position on the head. The electrodes should be set in such a way that current spans the brain. Electrodes should be placed ***between eye and ear on both sides of the head or one on the top of the head and the other under the jaw*** (*Grandin, 2010b*). Electrodes should never be placed on the neck or elsewhere than recommended, because the current will bypass the brain and stunning will fail (*Grandin, 2010b*).

Grandin (2001) found that placing the tongs in the wrong location was a major cause of return to sensibility. The importance of correct placement of electrodes for efficient stunning suggested also results of *Lammens et al. (2006)* who found that 1,8% of pigs was not effectively stunned due to incorrect placement of stunning tongs. *Spencer and Veary (2010)* observed proper placement of stunning tongs in 35-98% cases in five slaughterhouses. In study of *Dokmanovic et al. (2012)* was found high incidence of incorrect placement of stunning tongs (55-83%) in two abattoirs, which indicated serious problems in stunning practice (Table 1) and could be the cause of high incidence of unsuccessful stunning at first attempt (48-89%). This high incidence of improper electrode placement was due to lack of pig restraint as well as due to lack of staff training. It is therefore important that staffs are regularly trained regarding stunning procedure in order to improve treatment and welfare of pigs. Furthermore, most of small slaughterhouses in Serbia doesn't have restrainers why the optimum position of the electrodes is virtually impossible to attain under practical conditions and the practice of repeat application of electrical stunning is common (*Spencer and Veary, 2010*). In this cases it is recommended to apply current first to the head, to induce insensibility, and then immediately after to the side of the animal, right behind the front leg, to stop the heart (*Vogel et al, 2010*). Applying a second current to the chest abolished rhythmic breathing, righting reflex, eye tracking, vocalization (squealing), and natural spontaneous blinking (*Vogel et al, 2010*). After the electrodes are placed in the proper position, the current can be applied. Applying energized electrodes

(electrodes that direct electric current) on animal's body cause electric shocks and vocalizations in pigs. Such a practice, so-called "hot wanding", compromises animal welfare and is related to higher occurrence of blood splashes in meat. Hot wanding and vocalization during stunning was observed in 24-85% of pigs (*Dokmanovic et al., 2012*), which according to *Grandin (2010b)* indicated serious problems in stunning practice. In addition, electric tongs should hold firm on animal's body, since the interruptions of current application weaken its effect. Also, every time when current applies that leads to muscle contractions and blood splashes in meat (*Grandin, 2010b*).

Among proper electrode placement, it is necessary to pay attention to voltage, amperage and frequency of current at the time of stunning. According to the *Anon (2010)*, amperage and duration of stunning must be such as to ensure that animal instantaneously loses consciousness and remains unconscious until the occurrence of death. In order to induce instantaneous and painless loss of consciousness, it is necessary that amperage strong enough spans the brain and induces *grand mal* epileptic seizure. If insufficient amperage spans the brain, the animal will feel pain, electric shock or symptoms of a heart attack, and at the same time can be paralyzed (*Grandin, 2010b*). For these reasons, it is recommended that the amperage for pigs weighing approximately 100 kg is at least 1.25 amps, and for heavier pigs 2.0 amps or more (*Anon, 2007*). Therefore, it is necessary to sort pigs with similar live weight into groups and to regulate amperage according to live weight. Importance of amperage for successful stunning was determined by *Vegh et al. (2010)* who found that effective stunning in 88.3% cases was significantly related to amperage but not to other parameters such as voltage and duration of stunning. *Dokmanovic et al. (2012)* found that failure to stun was related to low current amperage (1.3 A), despite the fact that many pigs weighed more than 150 kg that required stronger amperage. The defined value of current amperage becomes achieved within the first second of application, and then it is necessary to apply 1-3 seconds more, in order to achieve effective stunning (*Grandin, 2010b*). *Dokmanovic et al. (2012)* observed that aplication of current lasted too long in one slaughterhouse (11.48 seconds) which could resulted in burns, blood splashes and lower pork quality. *Mikus et al. (2011)* found that application of electric current for at least 15 seconds in slaughterhouses instead of recommended 3 seconds was related to insufficient education of workers.

There must be sufficient voltage during electrical stunning to deliver the recommended minimum amperage. For pigs the recommended minimum voltage is 250 volts to insure insensibility (*Troeger, 1999*).

Today are very frequent stunning devices operating at 800 Hz, resulting in a better meat quality, reduced incidence of blood splashes and broken backs (*Grandin, 2010b*). *Berghaus and Troeger (1998)* evaluated animal welfare

implications of higher frequency (500 or 800 Hz) electrical stunning in comparison to "normal" (50 Hz) stunning and concluded that all stunning frequencies tested (50, 500, 800 Hz) caused an effective stun within a minimum current flow time (1.3 ampere constant) of 0.3 seconds. All tested stunning frequencies were in conformance with animal welfare demands. One disadvantage of stunning with too high frequencies is that the pigs regain sensibility more quickly than pigs stunned using frequencies of 50 to 60 cycles (*Anil and McKinstry, 1992*).

As was previously stated, in order to properly perform stunning procedure workers have to be careful, trained and competent. The equipment used for stunning should be in such a way designed, constructed and maintained as well as regularly cleaned and calibrated to ensure optimal current flow and effective stunning of pigs (*Anon, 2007*). Furthermore, electrodes must be cleaned frequently to insure that a good electrical connection occurs between stunner and animal.

Insensitivity during slaughter procedure

Properly stunned animals are not conscious and do not feel stimulations while hanging on the rail. A sign that pig is effectively stunned is occurrence of *grand mal* epileptic seizures that consists first of tonic, and then clonic convulsions (*McKinstry and Anil, 2004*). During epileptic seizure raise levels of neurotransmitters glutamate and aspartate, which lead to excessive neuron excitation when animal is not aware and sensitive to pain. Tonic muscle contractions last 10-15 seconds, when it is recommended to bleed the animal, and then start clonic spasms of extremities, in the form of walking, kicking or paddling, which last about 30 seconds (*McKinstry and Anil, 2004*).

Signs that an animal becomes aware and sensitive to pain are: 1) the rhythmic breathing (if ribs make at least two respiratory movements), 2) vocalization while hanging on the bleed rail, 3) spontaneous blinking, 4) the righting reflex, where the animal is trying to lift its head; 5) sensitivity to a pin prick on the nose (*Grandin, 2011b*). The occurrence of one or more signs of returning to consciousness after stunning is unacceptable from the point of animal welfare and none of the procedures should started before animals become re-stunned. After it is determined that the animal is effectively stunned, it can be slaughtered. According to Anon (2010), bleeding should be peformed as soon as possible after stunning and should be rapid, profuse and complete to prevent that animal regain consciousness. Pigs are usually bled by cutting the *truncus brachiocephalicus*. The pigs should be bled upto 15 seconds after stunning (*Anon, 2004*). This value is determined on the basis of experimental studies where first signs of sensitivity occur after 38 seconds (corneal reflex after 37 ± 12 seconds, rhythmic breathing after 44 ± 8 seconds and the righting reflex after 59 ± 15 seconds of stunning) (*McKinstry and Anil, 2004*)

and that the brain loses its function after 14 to 23 seconds of bleeding (*Gregory and Wotton, 1986*). In addition, this practice reduces the incidence of blood splashes in meat, because cutting the blood vessels reduces high blood pressure, which is caused by applied current. Problems with too long stun to stick interval were observed by *Dokmanovic et al. (2012)* and *Mikus et al. (2011)* who found that slaughter was on average performed after 72.48 and 69 seconds, respectively. These too long stunning to bleeding interval lead to regaining of sensibility and ***consciousness in pigs.***

In the study of *Dokmanovic et al. (2012)* was found high incidences of reflexes that ***indicated return of consciousness,*** 89% of pigs showed natural blinking, 9% rhythmic breathing, 61% righting reflex and 19% of pigs vocalized after stunning. This situation resulted from the incorrect placement of electrodes, insufficient amperage and too long interval from stunning to bleeding. Also insufficient amperage, insufficient duration of stunning, improper placement of stunning tongs and to too long interval from stunning to bleeding was reason for unsuccessful stunning of 15.6% of pigs (*Anil and McKinstry, 1992*). In all these cases it is necessary to re-stun pigs as soon as possible in order to ensure animal welfare.

Conclusion

In order to safe animal welfare as well as meat quality, it is important to perform stunning procedure in accordance with recommendations. The most common problems during stunning in our slaughterhouses are incorrect placement of stunning tongs, insufficient amperage and too long interval from stunning to bleeding. Therefore, staff training is necessary regarding stunning procedure in order to improve treatment and welfare of pigs. Furthermore, all stunning equipment should be maintained in good working conditions for achieving efficient stunning.

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Činioci od značaja za uspešnost omamljivanja

M. Dokmanović, M. Ž. Baltić, N. Karabasil, N. Glamočlija, S. Pantić, R.Marković, M. Todorović

Rezime

U Evrpskoj Uniji kao i u našoj zemlji postoje zakonske odredbe koje nalažu da životinje u toku klanja moraju biti nesvesne, ne smeju osećati bol i u takvom stanju moraju ostati dok ne nastane potpun gubitak moždanih funkcija zbog iskrvarenja. Tokom omamljivanja svinja strujom postoje dve kritične tačke za praćenje uspešnosti omamljivanja, a to je procenat životinja koje su efikasno omamljene pri prvom pokušaju i procenat životinja koje ostaju neosetljive tokom celog postupka klanja. Za uspešno omamljivanje neophodno je da radnici budu pažljivi, dobro obučeni i da pravilno izvode postupak omamljivanja. Osim toga, oprema koja se koristi za omamljivanje treba da bude tako projektovana, izgrađena, održavana, čišćena i redovno kalibrisana kako bi se osigurao optimalan protok struje i efikasno omamljivanje. Postupak omamljivanja utiče na dobrobit životinja, ali i na kvalitet mesa, stoga treba ovaj postupak izvoditi u skladu sa preporukama.

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RESULTS OF REPRODUCTIVE RESEARCH IN FEMALE MANGALICA PIGS

K. P. Brüssow[1], I. Egerszegi[2], J. Rátky[2]

[1]Leibniz Institute for Farm Animal Biology, Wilhelm-Stahl-Allee 2, 18196 Dummerstorf, Germany
[2]Research Institute for Animal Breeding and Nutrition, Gesztenyés ut. 1, 2053 Herceghalom, Hungary
Corresponding author: bruessow@fbn-dummerstorf.de
Short communication

Abstract: Since in 1996 the Hungarian Mangalica was endangered, a Hungarian-German project was activated to propagate Mangalica and to highlight the physiological background of low fecundity. The institute's breeding herd in Herceghalom was increased by means of adapted oestrus synchronization systems and embryo transfer (ET). Altogether 202 Mangalica piglets were born after ET. Research was focused on follicle and oocyte qualities and on uterine development during early pregnancy. Compared to Landrace, no differences in morphometric data of the reproductive organs, but altered intrafollicular oocyte maturation and restriction of uterine growth during early pregnancy were found, which could be reasons of lower fecundity in Mangalica. Similar release profiles of reproductive hormones FSH, LH, progesterone (P4) and estradiol were obtained during estrus cycle and early pregnancy. However, the intrafollicular P4 concentrations were 10-times higher in Mangalica which could have an influence on oocyte development. Considerable differences were found in the P4-secretion ratio per corpus luteum and in leptin concentrations. Both hormone concentrations were 3- to 4-times higher in Mangalica. The higher level and the up to one day later decrease of progesterone, which prolongs the negative feedback of P4 on gonadotropin secretion, and the increased leptin concentrations substantially down-regulated the pulsatile release of LH at the follicular phase of the estrus cycle. Thus the gonadotropin secretion was impaired, and as a consequence growth and maturation of follicles was diminished in Mangalica. Our research provided differences in the reproductive processes in Mangalica which could be estimably for the propagation of this native breed.

Key words: Mangalica pig, fecundity, oocyte development, reproductive hormone

Introduction

The Hungarian Mangalica, which belongs to the worldwide few fatty pig breeds, originated by crossing previous ancient pig breeds and the Serbian Sumadia in the Carpathian Basin from 1833 *(Enesei Dorner and Kovacsy, 1926)*. The earliest describes of Mangalica had mentioned two types, namely the White and Black Mangalica *(Czilchert, 1859)*. Of the later described five colour types (Blond, Black, Swallow-Belly, Brown and Red; *Kiss, 1926*) nowadays only the Blond, Swallow-Belly and Red Mangalica have been remained. They were recently determined by molecular genetic analysis to be three different breeds *(Zsolnai et al., 2006)*.

Mangalica is an extremely fatty pig breed. While adipose tissue is about 65-70% of the carcass, lean meat is less than 35%. Nevertheless consumers do agree that its meat is at the highest level and very tasty. Until the 1920s, different fat products (i.e. bacon and lard) were the main food of the average Hungarian people. Mangalica were also the flagship of Hungarian agriculture exports and about half a million fattened or slaughtered pigs were annually exported to European countries. Until the 2nd World War the Mangalica population was enormously growing and the Hungarian lard, bacon, sausage and salami were well known and acknowledged merchandises in Europe *(Enesei Dorner and Kovacsy, 1926; Rácz, 1932)*.

However, consumption habits changed and the need for lean pork increased considerably. An intensive crossing program with commercial breeds was ruled by the Research Institute for Animal Breeding during the 1950s to improve metabolic parameters, weight gain and pork quality suitable for different processed products *(Horn et al., 1952; Vincze, 1963)*. Nevertheless, in the 1950-ies the Mangalica stock rapidly reduced and, despite the former prosperous period, nearly disappeared in the 1970s. Only 34 breeding sows could be found in the herd book of 1975 *(Brüssow et al., 2005)* and the Mangalica breed was listed in the Red Data Book as endangered species. Fortunately, renaissance of Mangalica was beginning in the 1990s when governmental, scientific and private programs were commenced to preserve, propagate and exploit the unique Mangalica characteristics like adaptive ability to extreme and extensive climate and housing conditions, stress and disease resistance, motherliness and in particular excellent meat quality *(Egerszegi et al., 2003a)*. In 1996, a German-Hungarian research program started to propagate Mangalica pigs in the institute's breeding herd in Herceghalom by transferring embryos into Landrace sows. This initial program resulted in birth of 202 Mangalica piglets (Table 1). Furthermore, this program was aimed to study the physiological background of the low fecundity in Mangalica.

Table 1. Results of Mangalica embryo transfer 1996-2007

Years	No. of donors	No. of recipients	No. of transferred embryos	No. of Mangalica piglets born
1996-1999	59	31	625	164
2000-2007	31	12	190	38
Total	90	43	815	202

Looking at commercial aspects, a large project was started in 1991 under the name of "Real Mangalica" with special emphasis on in vivo gene preservation and development of premium quality meat products *(Tóth, 2005)* mostly by the appearance of a new market demand coming from Spain, the homeland of premium ham and other processed pork products.

Research on reproductive physiological characteristics

Ovarian function and uterine development

Mangalica pigs which were bred and kept in the past in extensive environments attained puberty at age of 15-16 month and at about 100 kg bodyweight, and revealed seasonal cyclic activity and farrowing. Typically, sows gave birth of 4-6 piglets, however in the 1930s, 20 % of the breeding sows farrowed always 6.5 to 7.5 piglets *(Blatz, 1938)*. Our research has been focused on the background of the modest prolificacy of Mangalica compared to modern (Landrace) breeds. There was only one report available to date about the number of ovulated follicles in Mangalica *(Bulatovici, 1932)*. Here, the number of ovulation was quite poor compared to Berkshire sows (9.9 *vs*. 12.4). In our studies in untreated gilts *(Rátky and Brüssow, 1998; Brüssow et al., 2004)*, the mean number of follicles/*corpora lutea* was 10.2 ± 2.5 (range: 6.8 to 11.7). However, the number of Graafian follicles could be increased when Mangalica were treated with exogenous gonadotropins (750 to 1250 IU equine chorionic gonadotropin, eCG), and the mean number of stimulated ovulation (18.2 ± 2.3) was comparable to that of commercial breeds *(Rátky and Brüssow, 1998; Rátky et al. 2001; Egerszegi et al. 2007)*. Consequently, Mangalica ovaries have the potential to grow a higher number of ovulatory follicles.

Observing ovarian luteal features and genital tract parameters of Mangalica gilts at slaughter, it was shown that cycling ovarian activity appeared at the age of 12 to 15 month. Furthermore, ovarian, oviductal and uterine weights, and oviductal and uterine lengths were in the range of Landrace gilts *(Brüssow et al., 2004)*. However, differences were observed in uterine morphology at the beginning of

pregnancy (Figure1). The length of both uterine horns were significantly shorter in Mangalica (124 ± 5 *vs*. 188 ± 6 cm; P<0.01) and afterwards uterus did not elongate (within days 1 to 24) in contrast to pregnant Landrace gilts where uterine horns were continuously growing. Uterine weight started to increase significantly later in Mangalica (from d 12 to d 24) compared to Landrace (from d 1 to d 12), respectively *(Brüssow et al., 2004)*.

Beside the lower number of ovulated follicles, the quality of ovulated oocytes could be a reason of lower fecundity in Mangalica. Significant morphologic differences of cumulus-oocyte-complexes confirmed different maturation periods in Mangalica gilts *(Egerszegi et al. 2001)*. Thus, the proportion of oocytes with compact cumulus was higher in Mangalica than in Landrace gilts (31 *vs*. 16%), but less oocytes had expanded cumulus (62 *vs*. 78%). The chromatin configuration of oocytes also differed between Landrace and Mangalica gilts, i.e. more matured oocytes (telophase I/metaphase II) were in Landrace sows (62 *vs*. 27%).

Summarizing, these studies strengthened the concept that apart from the diminished ovarian activity and oocyte development restricted uterine development may affect early pregnancy and can be an additional reason of lower prolificacy.

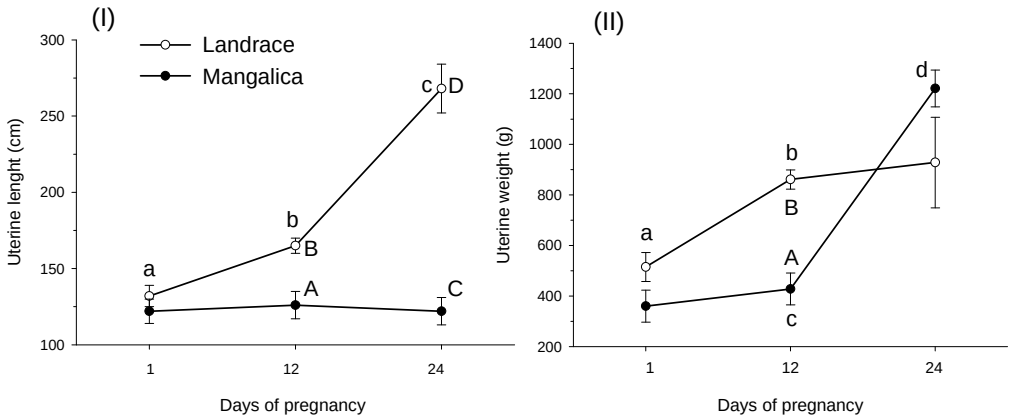

Figure 1. Dynamic of uterine length (I) and uterine weight (II) development in Mangalica and Landrace gilts on days 1, 12 and 24 of pregnancy *(Brüssow et al., 2004)*
(Data with different lower case P<0.01 within breed; with different capital letters P<0.01 between breeds)

Reproductive hormone levels

A series of experiments was performed to elucidate sexual steroid secretion during the oestrus cycle. Luteinizing hormone (LH), estradiol (E2), progesterone (P4) and leptin contents were analysed and compared to Landrace gilts *(Egerszegi*

et al., 2003b; Brüssow et al., 2008). Mangalica revealed similar patterns of steroid hormone secretion as Landrace gilts. However, despite the lower number of corpora lutea, both the preovulatory E2-peak (46.5 ± 5.7 *vs*. 26.0 ± 6.8 pg/ml) and the P4 concentrations during the luteal phase (12.9 ± 2.6 *vs*. 9.3 ± 2.2 ng/ml) were higher in Mangalica. This was confirmed in our recent study when a chronic catheter was inserted in the *V. cava cranialis* for blood sampling for better understanding of ovarian steroid secretion (*Brüssow et al., 2008)*. In Landrace gilts, the number of LH pulses (2.0 to 3.3 pulses), LH pulse amplitudes (0.61 to 0.65 ng/ml) and basal LH concentrations (0.04 to 0.1 ng/ml) were unchanged between day 9 and day 15. In Mangalica gilts, however, the measured LH concentrations were always below the sensitivity of the LH assay (0.03 ng/ml) on days 9 to 15, and no pulses were recorded. Measurable LH (mean concentrations: 0.13 ng/ml) was found in Mangalica only from days 16 to 19 at concentrations that were similar to Landrace (0.10 ng/ml). Because the M gilts had a lower number of corpora lutea than Landrace (9.7 ± 2.3 *vs*. 20.5 ± 4.4), an influence of breed ($P<0.01$) was determined based on the ratio of P4 secretion per corpus luteum. The mean ratio was about four times higher in Mangalica (Table 2).

Table 2. Mean (± SE) number of *corpora lutea* (CL), and mean concentrations of P4 and P4 per CL (ng/ml) in the *Vena cava caudalis* of Mangalica (n = 3) and Landrace gilts (n = 4) from day 7 to 19 of the oestrous cycle *(Brüssow et al., 2008)*

Breed	Number of CL	P4 concentration (ng/ml)	P4 concentration per CL (ng/ml)
Mangalica	9.7 ± 2.3^{a}	75.8 ± 2.9^{a}	8.7 ± 0.7^{a}
Landrace	20.5 ± 4.4^{b}	49.6 ± 4.2^{b}	2.8 ± 0.2^{b}

^{a,b} $P<0.01$

In Mangalica, the P4 concentrations were always significantly higher during the oestrus cycle, and the P4 level dropped down about two days later compared to Landrace gilts (Figure 2a). The concentrations of leptin significantly ($P<0.05$) differed during the oestrus cycle, too (Figure 2b).

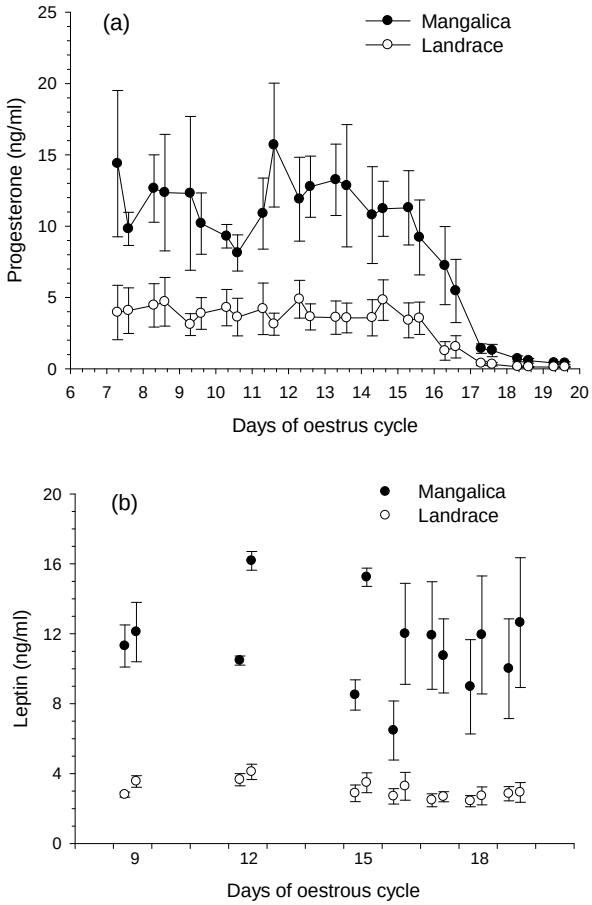

Figure 2. Mean concentrations of P4 per CL (a) and leptin (b) in Mangalica and Landrace gilts during the oestrus cycle *(Brüssow et al., 2008)*

Interestingly, follicular steroid hormone milieu differed considerably in the two breeds *(Egerszegi et al., 2007)*. The concentrations of E2 were 1.7-times (30.3 ± 8.6 *vs*. 17.4 ± 6.7 pg/ml) and that of P4 5-times higher (1766.3 ± 613.7 *vs.* 342.5 ± 119.8 ng/ml) in Mangalica follicular fluid. Additionally in this experiment, feeding high (2.5 kg) or low (1.25 kg) energy level had no influence on steroid hormone levels and oocyte quality in both breeds, but had an impact on the number of preovulatory follicles. However, only in Landrace gilts the restriction of energy lowered the number of follicles (32.3 ± 10.5 *vs.* 17.1 ± 12.3, $P<0.05$), whereas the

follicular development in Mangalica was not affected (25.3 ± 2.9 *vs.* 28.8 ± 7.3). This underlines the adaptive characteristics of Mangalica.

Conclusion

Based on our research data it can be concluded that several factors are involved to be the physiological background of the low fecundity in Mangalica. The higher level of systemic P4 concentrations, despite lower number of corpora lutea, and the later drop of P4 at the beginning of the follicular phase of the oestrus cycle together with an increased leptin secretion consequently alter the LH secretion pattern in Mangalica. This again, can influence cyclic follicle recruitment and explain the lower follicle number. The unexpected high intra-follicular steroid hormone concentrations could be responsible for the altered oocyte maturation in this breed. Furthermore, growth restricted uterine development may influence the initial process of early pregnancy and be another reason of low fecundity in Mangalica.

Rezultati istraživanja reproduktivnih performansi ženskih svinja rase mangulica

K.P. Brüssow, I. Egerszegi, J. Rátky

Rezime

Od 1996. mađarska mangulica je ugrožena, mađarsko-nemački projekat je aktiviran da promoviše Mangulicu i da ukaže na fiziološku pozadinu niske plodnosti. Stado priplodnih grla Instituta u Herceghalomu povećano je putem sistema sinhronizacije estrusa i embriotransfer (ET). Ukupno 202 prasadi rase mangulica su rođeni posle ET. Istraživanje je fokusirano na folikule i kvalitet jajnih ćelija i na razvoj materice tokom rane trudnoće. U odnosu na landrasa, nema razlike u morfometrijskim podatacima reproduktivnih organa,ali izmenjeno sazrevanje intrafolikularnih oocita i ograničavanje rasta materice tokom rane trudnoće su utvrđeni, što bi moglo da bude razlog niže plodnosti kod mangulica. Sličan profil otpuštanja reproduktivnih hormona FSH, LH, progesterona (P4) i estradiola su dobijeni tokom estrus ciklusa i rane trudnoće. Međutim, intrafolikularna P4 koncentracije je 10 puta veća kod mangulica koje mogu imati uticaj na

razvoj jajne ćelije. Značajne razlike se javljaju u izlučivanju P4- odnosu prema corpus luteumu i u koncentracijama leptina. Koncentracije oba hormona su 3 - do 4 puta veće kod mangulice. Viši nivo i kasnije smanjenje progesterona za jedan, koji produžava negativnu reakciju P4 na lučenje gonadotropina, i povećana koncentracija leptina značajno utiču na otpuštanje LH u folikularnoj fazi estrus ciklusa. Tako je narušeno lučenje gonadotropina, a kao posledica, rast i sazrevanje folikula je umanjeno kod rase mangulica. Naše istraživanje utvrdilo je razlike u reproduktivnim procesima svinja rase mangulica koji bi mogli biti značajni za razmnožavanje ove autohtone rase.

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THE ISOLATION OF LISTERIA MONOCYTOGENES FROM RBACON

J. Kureljušić[1], N. Jezdimirović[1], V. Vuković[1], D. Jakić-Dimić[1], S. Vesković-Moračanin[2], M. Jezdimirović[3]

[1]Institute of Veterinary Medicine of Serbia, Auto put 3, 11000 Belgrade, Republic of Serbia
[2]Institute of meat hygiene and technology, Kaćanskog 13, 11000 Belgrade, Republic of Serbia
[3]Faculty of veterinary medicine, Bulevar oslobođenja 18, 11000 Belgrade, Republic of Serbia
Corresponding author: jasnakureljusic@yahoo.com
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Abstract: Many different pathogens can be found on meat and meat products, including *Escherichia coli* 0152:H7, *Salmonella spp*., *Listeria monocytogenes* and *Staphylococcus aureus*. *Listeria monocytogenes* is foodborne pathogen that cause listeriosis in animals and humans. Listeriosis is very severe disease with an estimated case fatality rate of 20-40% in humans, considerably higher than most other foodborne pathogens. The presence of *Listeria monocytogenes* in food is a critical public health concer. Listeria monocytogenes can grow and survive during storage at refrigeration or mildly abusive storage temperatures, thus contribute to the burden of food borne listeriosis associated with the consumption of contaminated food. This organisam can survive under relatively extreme physicochemical conditions such as low temperature (as low as – 0,4^{o}C), and high salt content. Pathogen may be found in live animals and may contaminate the meat if cross contamination occurs during the slaughter, or they may colonize the intestines of animals, which could contaminate muscles at slaughter. Thorough cooking or processing of meat product should destroy vegetative pathogens, but contamination of cooked meat products can occur from the environment, after processing and before packaging in the processing plant. In this work the presence of *Listeria monocytogenes* in 90 samples of bacon was determined using horizontal method for the detection and enumeration of *Listeria monocytogenes* (SRPS EN ISO 11290-1 and SRPS EN ISO 11290-2). *Listeria monocytogenes* was found in one sample of bacon. This finding can be consequence of contamination during processing at slaughter or after processing and before packaging in the processing plant. Further, contamination or microbial growth can occur through improper handling during storage, at retail or at the home. Organisms can grow to large numbers if time and temperature controls are not in place.

Listeria monocytogenes poses a public health concern and risk management actions are required to reduce the levels of listeriosis that currently exist. Therefore, food safety management strategies must be in place to protect public health.

Key words: *Listeria monocytogenes,* bacon, isolation

Introduction

L.monocytogenes is a facultative intracellular pathogen that is able to invade and survive within mammalian cells, including macrophages and several human tissue culture cell lines. This organism can be isolated from soil, water, sewage and vegatation and as part of fecal flora of wide variety of animals *(Farber and Peterkin, 1991)*.

Listeria monocytogenes is also a common contaminant in food products. The organisam is able to grow in biofilms on the surface of various foods, and refrigeration actually augments further organism growth at 4^{o}C. It is catalase positive and oxidase negative and expresses β hemolysin which produces zones of clearing on blood agar. The hemolysin acts synergistically with the β hemolysin of *Staphylococcus aureus* on sheep erythrocytes, the substance mediating this effect is known as the CAMP factor. The organism posesses peritrichous flagell, which give it a characteristic tumbling, motility, occurring only in a narrow temperature rang. When the organism is grown between 20 and 25^{o}C, flagellin is both produced and assembled at the cell surface, but at 37^{o}C flagellin production is markedly reduced. The colonies demonstrate a characteristic blue-green sheen by obliquely transmitted light. Because of their ubiquity in foods, humans probably have contact with these organisms on daily basis and as result some individuals become fecal carriers of *L. monocytogenes (Winn et al., 2006)*.

L. monocytogenes is foodborne pathogen that can cause listeriosis. Listeriosis is a very severe disease with an estimated case fatality of 20-40%, considerably higher than most other foodborne pathogens. In adults, listeriosis occurs in an invasive or noninvasive form *(Wals, 2006)*. After initial flu-like symptoms (fever, fatique, malaisa, cramps, vomiting and diarrhea), invasive listeriosis in adults is characterized by the onset septicemia and meningitis. In a pregnant woman, invasive listeriosis can lead to spontaneous abortion. Invasive listeriosis typically occurs in susceptible individuals who have one or more underlying conditions that depress immune function, which predispose them to this disease.

The growth of *L. monocytogenes* in foods is dependent on the intrinsic characteristics of the product (e.g. pH, water activity), the extrinsic characteristics of the product (e.g. storage temperature, relative humidity) and processing techniques (e.g. cooking, non-thermal processing) used in its production. The principal factors that influence the survival and growth of *L. monocytogenes* in food are temperature, pH and water activity. As with other bacteria, the tolerance of *L. monocytogenes* to particular environmental constraints (processing and/or storage conditions) is greatest when all other conditions are optimal for growth. However, it has also been demonstrated that previously stressed cells (e.g. exposure to sub lethal heating before process heating) can be more resistant to additional stresses *(Winn et al., 2006)*.

Hurdle technology refers to the concept of achieving control of a risk of contamination of food by combining in series, a number of measures that would not individually be adequate for control. Each individual control measure is considered a hurdle to the survival and growth of pathogens. A hurdle may be based on temperature (e.g. cooking), (e.g. drying, adding salt/sugar), acidity (e.g. pickling), redox potential (e.g. fermentation), preservatives (e.g. adding salt) and other measures *(Wals, 2006)*.

Materials and Methods

In this work the presence of *Listeria monocytogenes* in 90 samples of bacon was determined using horizontal method for the detection and enumeration of *Listeria monocytogenes* (SRPS EN ISO 11290-1 and SRPS EN ISO 11290-2). *Listeria monocytogenes* was found in one sample of bacon. Sampling was done according to prescribed methods (*Microbiology of food and animal feeding stuffs — Horizontal method for the detection and enumeration of Listeria monocytogenes — Part 1: Detection method and Part 2: Enumeration method)* In the laboratory, bacons were prepared and subsequently inoculated on culture medium in accordance with SRPS EN ISO standards under the Regulation on the general and special conditions of food hygiene at any stage of production, processing and transport *(Official Gazette 72/10)*.

Results and discussion

It was examined a total of 90 samples of bacon, and *Listeria monocytogenes* was found in one sample (0.90%). *L. monocytogenes* is considered to be one of the most important agents of food-borne disease. Possible explanations for the emergence of human food-borne listeriosis as a major public health concern

include major changes in food production, processing and distribution, increased use of refrigeration as a primary preservation means for foods, changes in the eating habits of people, particularly towards convenience and ready-to-eat foods, and an increase in the number of people considered to be at high risk for the disease (elderly, pregnant women, newborns, immunocompromised).

It is not surprising that *L. monocytogenes* is found in meat and meat products when this considers at the natural habitat of this organism. Its widespread occurrence in nature, especially on plants, and its association in nature with other bacteria that are well established in meat makes it reasonable it finding in meat. Although it is not among the isolates reported in the early microbiological literature on meat here is no reason to believe that it has not always been in our meat supply *(Hereu et al., 2012)*.

Food samples intended for analysis must be representative, including the outer surface and the deep cut. No single procedure can be credited with being sensitive enough to detect *L. monocytogenes* from all types of food. In addition, sublethally injured *L. monocytogenes* cells can be found in processed food due to freezing, heating, acidification and other types of chemical or physical treatment. These sublethally injured bacteria require special culture conditions for damage repair, before being able to be detected in culture *(Taormina and Dorsa, 2010; Xi et al., 2012)*. It is possible that survival of *L.monocytogenes* in latter weeks of storage at non frozen temperatures indicates on more resistant subpopulation of cells. Attempts were made to raise an inoculums with a higher proportion of hardly, resistant cells than might otherwise be expected by growing *L.monocytogenes* at 10^0C over 2 weeks rather than 35^0C for 1-2 days *(Taormina and Dorsa, 2010)*.

However, the most feasible and practical means to reduce the risk of listeriosis in humans is through dietary and food preparation measures that not only decrease the risk of acquiring listeriosis, but also contribute to the prevention of other common food-borne infections such as those caused by *Escherichia coli* O157:H7, *Salmonella* and *Campylobacter (James, 1996)*. These preventive measures include thorough cooking of raw food of animal origin, keeping uncooked meats separate from vegetables, cooked foods, and ready-to-eat foods, thoroughly washing raw vegetables before eating, washing hands, knives, and cutting boards after handling uncooked foods, and avoiding unpasteurised milk or products made from it. Immunocompromised persons, pregnant women and other groups at increased risk of listeriosis should avoid foods that have been epidemiologically linked to this disease, e.g. soft cheeses. These individuals should also avoid other ready-to-eat foods, unless they are heated until steaming hot before being consumed *(Farber and Peterkin, 1991)*.

Conclusion

This finding can be consequence of contamination during processing at slaughter or after processing and before packaging in the processing plant. Further, contamination or microbial growth can occur through improper handling during storage, at retail or at the home. Organisms can grow to large numbers if time and temperature controls are not in place.

The food industry and public health agencies play a pivotal role in the prevention of food-borne listeriosis by developing and implementing effective HACCP programmes to reduce the presence of *L. monocytogenes* at all critical points in the food production and distribution chain (from the farm to the market).

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Nalaz *listeria monocytogenes* u slanini

J. Kureljušić, N. Jezdimirović, V. Vuković, D. Jakić-Dimić, S. Vesković-Moračanin, M. Jezdimirović

Rezime

Različiti patogeni se mogu naći u mesu i mesnim prerađevinama uključujući *E. coli* O152:H7, *Salmonella* spp., *Listeria monocytogenes* i *Staphilococcus aureus*. *Listeria monocytogenes* je jedan od patogena koji preko alimentarnog trakta može izazvati listeriozu životinja i ljudi. Listerioza je veoma ozbiljna bolest kod koje se stopa letaliteta kod ljudi kreće od 20 do 40 %, što je znatno više u odnosu na druge alimentarne patogene. Prisustvo *Listeria monocytogenes* u hrani predstavlja problem za javno zdravlje s obzirom da može da se umnožava i da opstane tokom skladištenja i hlađenja hrane. Ovaj mikroorganizam može da preživi pod relativno ekstremnim fizičko-hemijskim uslovima kao što je niska temperatura (niža od – 0,4^{o}C), i visok sadržaj soli. Patogeni se mogu naći na živim životinjama i mogu kontaminirati meso usled unakrsne kontaminacije tokom klanja, ili mogu da kolonizuju digestivni sistem životinja što takođe može dovesti do kontaminacije mesa. Pravilno kuvanje i prerada proizvoda od mesa bi trebala da inaktiviše vegetativne oblike ovog patogena, ali se kontaminacija kuvanog mesa može desiti i kasnije iz same okoline, nakon obrade u objektima za preradu mesa. U ovom radu prisustvo *Listeria monocytogenes* je ispitivano u 90 uzoraka slanine korišćenjem horizontalne metode

za detekciju i brojanje *Listeria monocytogenes* (SRPS EN ISO 11290-1 and SRPS EN ISO 11290-2). *Listeria monocytogenes* je nađena u jednom uzorku slanine. Ovakav nalaz može biti posledica kontaminacije tokom obrade na klanici, ili posle obrade i pre pakovanja u objektima za preradu. Nadalje, kontaminacija i rast mikoorganizama se može javiti zbog neadekvatnog skladištenja u objektima za promet ili u domaćinstvu. Njihovo intenzivno umnožavanje je omogućeno u uslovima kada temperatura skladištenja nije pod kontrolom. *Listeria monocytogenes* predstavlja veliki problem za javno zdravlje, tako da je upravljanje rizikom od ključnog značaja za smanjenje mogućnosti za pojavu listerioze. Iz tih razloga strategije bezbednosti hrane moraju omogućiti zaštitu javnog zdravlja.

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CARCASS QUALITY AND MEAT OF FATTENING PIGS SIRES LANDRACE AND YORKSHIRE

V. Vidović[1], A. Stoisavljević[2], D. Lukač[1], M. Stupar[1]

[1]Faculty of Agriculture, Department of Animal Science, Trg Dositeja Obradovića 8, 21 000 Novi Sad, Republic of Serbia
[2]PTK Panonija, Trg Lenjina 1, 24330 Panonija, Republic of Serbia
Corresponding author: vitomir.vidovic@stocarstvo.edu.rs
Original scientific paper

Abstract: The objective of this study was to determine the influence of the genotype sires on carcass quality and meat. The research was carried on 18 fattening pigs hybrid obtained by crossing F_1 sows with three boar Landrace and Yorkshire breeds. Each sires has had three offspring different body weight, 100 to 125 kg. The percentage meat on the carcass fattening pigs sires Yorkshire was 56.67%, and fattening pigs sires Landrace was 55.79%. Not established significant differences in the proportion of parts of carcasses between the offspring of these two breeds, considering that this is a fertile two breed with the same selection criteria. The analysis of variance also showed that breed did not influence the ratio of certain parts of the carcass. Between fattening pigs sires breed Yorkshire and Landrace there were no significant differences in the technological and nutritional quality indicators carcass, and that sire breed did not influence the technological and nutritional quality meat. The percentage of protein in meat as one of the most important indicators of the quality of meat, in fattening pigs both breeds is was about 20%. In all fattening pigs, muscle pH was within the normal range of 5.4 to 5.8., i.e. we had a "normal meat" without the appearance of PSE and DFD meat.

Key words: carcass quality, meat quality, sire breed

Introduction

On the market today, place all higher demands in terms of quality of pig carcasses and meat. Possible to predict the quality of pig meat is one of the crucial and key role in the manufacturers's business success, so genetic basis is the primary criterion in setting production goals (*Chan et al., 2002; Vidović et al., 2011*). On the quality pork carcass and meat influence genetic factors *(Affentranger at al., 1996; Senčić et al., 2003; Miller et al., 2000*) and numerous Paragenetic factors (*Therkildsen et al., 2001; James et al., 2002; Chiba et al., 2002*), including the

final body weight of fattening pigs (*Cisneros et al., 1996; Čandek-Potokar et al., 1998; Weatherup et al., 1998*). Optimal final body weight of fattening pigs depending on customer requirements, the needs of the manufacturing industry, the economy of production, but also the genetic potential of pigs for meat production. Genotype pigs with food, the greatest influence on fattening and meatiness fattening hogs (*Miller et al., 2000; Chiba et al., 2002*). Breeding pigs is trying to achieve the effect of heterosis for important production traits (*Vidović, 1987; Campbell et al., 1988; Čandek-Potokar et al., 1988; Petričević et al., 2000*). Finding the the best combination of of crossing is a continuous process, due to the fact that the selection of individual genes frequency changes continuously. Growth traits are controlled by additive genes on the middle level, while the ratio of tissue and share in the structure of carcasses belong in the highly heritable. This selection has no same value.

The consumers' interest in pork and pork products is maintained and originated upon many factors. Composition of pork and thereby its nutrition value, sensoric perception, especially its fine taste, softness, crispness and juiciness plus variety of culinary preparations may serve as the examples (*Lamb, 1994*). As presented by *Wood et al. (1994)*, *Jeleníková (2003),* a consumer considers meat having an optimum composition regarding its nutritious value to be a quality meat. Meat is a very rich and universal source of nutrients (*Ruprich, 2003*). Primary importance of meat is based in protein content especially (*Lawrie, 1998*). Rating the quality of pigs and price formation at the slaughter line prerequisite to the establishment of trust between farmers and slaughter industry. The motive of these relationships is profits, because selection for meatiness farmers make more money savings on food, while slaughterhouse will buy animals with more meat of optimum quality.

Materials and Methods

Dissected pigs are with one commercial farm in Vojvodina. Dissection was performed in 18 hybrid fattening pigs by crossing the F_1 sows with three of boars Landrace and Yorkshire breeds. Any father has had three offspring different body weight, 100 to 125 kg. Composition and quality food used during the fattening period was the same for all animals. In the slaughterhouse for each offspring-fattening pigs fortified his sires genotype, sex, animal live weight, the weight of the hot and cold carcass, three measures of back fat (withers, back and rump in the middle *m. gluteusa mediusa*), carcass length, yield and the proportion of meat in the carcass. After that, is done a total pig carcass dissection and measurement of ratio of certain carcass parts. The samples for analizy technological and nutritional

quality of meat (protein levels, water, intramuscular fat, minerals, muscle pH) were taken with four anatomical locations carcass: neck, shop, shoulder, ham.

After the data obtained on the slaughter line and laboratory analysis, using is method of analysis of variance in order to determine genetic differences between different genotypes of sires. Variation statistics were used to analyze the variability of the traits between groups and test for of significance differences.

Results and Discussion

Table 1 shows values of of carcass quality of fattening pigs sires Yorkshire and Landrace. The average body weight at slaughter fattening pigs fathers Yorkshire was about 114 kg, and fattening pigs sires Landrace about 117 kg. Warm carcass weight of fattening pigs paternal Landrace was higher (93.57 kg) of the fathers Yorkshire fattening pigs (86.52 kg) about 4 kg but they had a higher cooling loss for 1.57% of fattening pigs fathers Yorkshire. In research *Kosovac et al. (1998),* the average weight of warm carcass fattening pigs pure breeds, double crosses was from 80.96 to 88.84 kg. *Senčić et al. (2003)* in his research they received warm carcass weight average of the 79.82 to 81.66 kg, which is much less compared to the weight of carcasses obtained in this study.

By *Čepin and Čepon (2001)* in the modern production of all aforementioned factors, animal nutrition is the most important impact on carcass quality, while genetic effects explain only 25-30% of the total variability in carcass quality. On the effect of genotype on the meatiness pig carcasses reported *Petričević et al. (2000)* and *Kralik et al. (2004),* while about the importance of choice boars for getting meaty of boars fattening pigs *Kušec et al. (2003). Ball et al. (1996)* have pointed out that influence of breed may vary considering the area of swine production, but also between different vendors in the same area for different animal husbandry intervention, depending on the selection goals.

Table 1. Parameters of the quality value of carcass of fattening pigs sires breed Yorkshire and Landrace

Parameters	Sire breed	$\bar{X}$	$\delta_{\bar{x}}$	δ	δ^2	V,%
Slaughter weigh, kg	Yorkshire	113.67	3.23	9.70	94.00	8.53
	Landrace	116.11	3.03	9.09	82.61	7.83
Weight of warm carcass sides, kg	Yorkshire	89.35	2.92	8.75	76.50	9.79
	Landrace	93.57	2.30	6.90	47.61	7.37
Weight of cold carcass sides, kg	Yorkshire	86.52	2.80	8.40	70.54	9.71
	Landrace	89.17	2.50	7.51	56.33	8.40
Loss cooling, %	Yorkshire	2.83	0.55	1.66	2.77	58.74
	Landrace	4.40	0.75	2.26	5.09	51.31
Randman, %	Yorkshire	79.41	0.80	2.40	5.77	3.02
	Landrace	80.18	0.75	2.25	5.05	2.80

After obtained data on the slaughter line, was performed pigs dissection and determination of ratio of certain body parts in the carcass fattening pigs (Table 2). The biggest and most important part of the carcas is ham with the proportion of about 31% in both breeds finishing pigs. Similar proportion received *Lukač et al. (2012a)* who getting in dissection of 29-30% share of ham in carcasses of fattening pigs Landrace and Yorkshire, *Kušec et al. (2008.)* which received about 25% sthare of ham in carcasses. Of similar share were given *Latorre et al. (2004.), Petričević et al. (2000), Pulkarabek et al. (2006), Ukmar et al. (2008).* After the ham, third of front (neck, blade, shank) makes up the largest share of the hull, about 26%, then the ribs with bacon about 16% and loin about 15% in both groups of fattening pigs. With offspring both breeds are not established significant difference in the share of of body parts in the carcasses between the breeds, considering that this is a fertile two breeds with the same selection criteria. *Hamilton et al .(2001.)* have determined only a significant effect terminal breed on the weight of pig carcasses and parts when shares are used in the crosses.

The analysis of variance showed that there was a statistically significant difference ($p<0.05$) between fattening pigs sires breed Yorkshire and Landrace in the share of heads, third of front (neck, blade, shank) and ham in carcass, while in other parts there were no difference ($p>0.05$). Also, analysis of variance showed that breed did not influence the shares of parts in the carcasses.

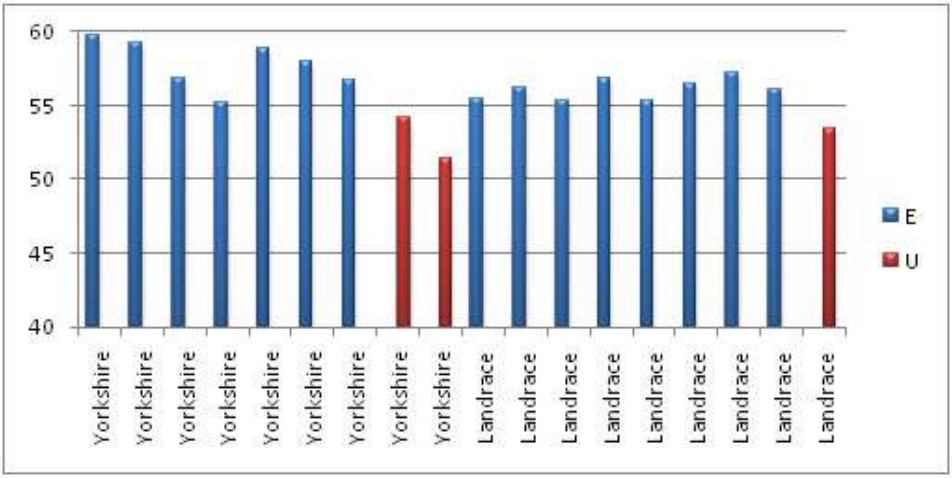
Table 2. Proportion of major parts in the pork carcass of fattening pigs sires breed Yorkshire and Landrace

Parameters	Sire breed	$\bar{x}$	%
Head, kg	Yorkshire	4.22^{b}	4.88
	Landrace	4.12^{a}	4.62
Anterior legs, kg	Yorkshire	0.87^{a}	1.00
	Landrace	0.90^{a}	1.01
Posterior legs, kg	Yorkshire	1.08^{a}	1.25
	Landrace	1.16^{a}	1.30
Jowl, kg	Yorkshire	2.52^{a}	2.91
	Landrace	2.84^{a}	3.18
Loin, kg	Yorkshire	13.43^{a}	15.53
	Landrace	13.24^{a}	14.85
Fillet, kg	Yorkshire	0.84^{a}	0.98
	Landrace	0.83^{a}	0.93
Third of front (neck, blade, shank), kg	Yorkshire	22.70^{b}	26.24
	Landrace	23.61^{a}	26.47
Ham, kg	Yorkshire	26.69^{b}	30.86
	Landrace	27.65^{a}	31.01
Ribs with bacon, kg	Yorkshire	14.13^{a}	16.34
	Landrace	14.83^{a}	16.63
Total, kg	Yorkshire	86.49	100.00
	Landrace	89.17	100.00

p < 0,05 – different small letters; p > 0,05 - same small letters

Percentage of muscle tissue in the carcass and carcass classification in the merchant class is shown in the graph 1. The average meatiness of fattening pigs sires Yorkshire was 56.67%, while the average meatiness of fattening pigs sires Landrace was 55.79%. Of the total number of carcasses, 83.33% of carcasses of E commercial classes, where the legislation classifies the carcases meatiness from 55 to 60%, while the 16.66% of carcasses classified in the U commercial classes, where the legislation classifies the meatiness of the carcases from 50 to 55%.

In research *Okanović et al. (2006)*, percentage of meat in 217 carcasses was about 56.20%, and ranged from 48.09 (R class) to 62.61% (S-class), respectively meat yield was between 37.23 and 48.58 kg. The largest number of carcasses (41.94%) was the E-class (55-60% meat), minimum (10.14%) were class R carcasses (45-50% meat), carcasses with the finest quality S class (> 60% meat) was 20.28%, while the carcasses U class (50-55% meat) was 27.65%. Therefore, about 80% carcasses had more than 50% meat, and belonged to the best, S, E and U categories.



Graph 1. Percentage of muscle tissue in the carcass and distribution of carcasses according to (S)EURO standard

As alleged by *Petrović et al. (2009),* the average percentage of meat in the carcasses of pigs in Denmark, Belgium and France is 60%, Spain 58%, Germany 56.7%, Sweden 57%, Poland 50%. Hungary 53%, Slovakia 52%, Norway 56%, Slovenia 55.4%.

Research in Slovenia in the period of 1996. to 2004. have shown that meatiness their pig population has increased in this period. Meat content in the carcasses 1996 year was 51.9%, but in 2004. increased to 55.9%. As a consequence, carcass were classified in the the S and E classes. While in 1996. in these two classes was entered 21.3% of pig carcasses, 2004. this percentage increased to 58.2%. This increase meat content in pig carcasses in Slovenia occurred because the prices of carcasses been formed at the basis of meatiness on the slaughter line, and partially because of better management of the farm, and because of the increased use of Pietrain sires in the production of hybrids (*Čandek-Potokar et al., 2004*).

According to *Vidovic et al. 1987*, all sows must have Minim 55% meat on the carcass, and the F_1 sows, which are used in breeding programs with obtaining two-breed hybrid lines, in order improve the quality of pig carcasses F_1 generation, it must be achieved by further selection and a better selection of pure breeds. About the importance the choice of breeds of boars in breeding in order to achieve greater percentage of meat fattening pigs show results *Kušec et al.(2003), Kralik et al. (2004)*. In research *Lukač et al. (2012b)* comparing the share of muscle tissue in the carcass in the breed was not statistically significant difference ($p>0.05$) between the two fertile breed (Landrace and Yorkshire) while the there is a statistically significant difference ($p<0.05$) between the two fertile breed and terminal breed Hampshire. The same results were obtained *Kušec et al. (2003), Petričević et al. (2000)* and *Vidović (1987)* who found a statistically significant difference between fertile and terminal breed.

In table 3 presented are technological and nutritive characteristics carcasses fattening pigs. From table we can see that there is no statistically significant difference ($p> 0.05$) between the fattening pigs sires breed Yorkshire and Landrace in technological and nutritional quality indicators carcasses. Also, analysis of variance was found to sire breed did not influence the technological and nutritional quality was fattening pigs carcass ($p> 0.05$).

Table 3. Technological and nutritional quality of carcasses of fattening pigs sires breed Yorkshire and Landrace

Parameters	Sire breed	$\bar{X}$	$\delta_{\bar{X}}$	δ	δ^2	V,%
Contents of protein,%	Yorkshire	20.02[a]	0.36	2.18	4.77	10.88
	Landrace	19.77[a]	0.32	1.92	3.71	9.71
Contents of water,%	Yorkshire	70.37[a]	0.67	4.06	16.52	5.76
	Landrace	70.46[a]	0.54	3.28	10.77	4.65
Contents of ntramuscular fat,%	Yorkshire	7.11[a]	0.94	5.69	32.44	80.02
	Landrace	7.13[a]	0.80	4.85	23.56	68.02
Contents of the relative protein content of connective tissue,%	Yorkshire	4.13[a]	0.38	2.29	5.26	55.44
	Landrace	4.22[a]	0.40	2.40	5.76	56.87
Contents of mineral substances,%	Yorkshire	2.48[a]	0.05	0.30	0.09	12.09
	Landrace	2.62[a]	0.04	0.25	0.06	9.54
pH muscules	Yorkshire	5.50[a]	0.03	0.20	0.04	3.63
	Landrace	5.45[a]	0.03	0.18	0.03	3.30

$p > 0,05$ - same small letters

The percentage of protein in meat as one of the most important indicators of the quality of meat, in fattening pigs both breeds is was about 20%. In recent years, the high consumer demand for low-fat pork has increased the production of lean genotype pigs. The genetic potential for maximum protein accretion and the slope of the linear response phase of protein accretion generally varies in modern commercial pigs (*Campbell and Taverner 1988; Gatel and Grosjean 1992*). *Sather et al. (1991)* reported that the select lines of Landrace and Yorkshire pigs currently produced by pig breeding companies had generally superior growth rate and carcass quality than the pigs tested across Canada at Record of Performance test stations. According to *Vidović et al. (2011)* a good meats is considered one that has a minimum of 23% protein and about 2.5% intramuscular fat. The protein content in the meat of pigs Landrace and Yorkshire from 25 - 30 kg of body weight is average 26%. This result has been achieved in pigs selection of pure breed Landrace, Yorkshire and Duroc. The water level in the meat of fattening pigs both fathers breed was about 70%, where the on the basis of the literature we can conclude that it was within normal limits. According *Žlender (1997)* and many other authors, fresh pork meat contains about 75% water and its content is the prevalent inter - and intracellular meat ingredient. Percentage of intramuscular fat

in the meat of fattening pigs of both breeds was about 7%, while the percentage of minerals was between 2.48 with fattening pigsYorkshire and 2.62% of fattening pigs Landrace. According *Žlender (1997)* and many other authors, fresh pork meat contains about 1% of mineral substances. In all fattening pigs, muscle pH was within the normal range of 5.4 to 5.8., i.e. we had a "normal meat" without the appearance of PSE and DFD meat.

Conclusion

The percentage meat on the carcass fattening pigs sires Yorkshire was 56.67%, and fattening pigs sires Landrace was 55.79%. Not established significant differences in the proportion of parts of carcasses between the offspring of these two breeds, considering that this is a fertile two breed with the same selection criteria. The analysis of variance also showed that breed did not influence the ratio of certain parts of the carcass. Between fattening pigs sires breed Yorkshire and Landrace there were no significant differences in the technological and nutritional quality indicators carcass, and that sire breed did not influence the technological and nutritional quality meat. The percentage of protein in meat as one of the most important indicators of the quality of meat, in fattening pigs both breeds is was about 20%. In all fattening pigs, muscle pH was within the normal range of 5.4 to 5.8., i.e. we had a "normal meat" without the appearance of PSE and DFD meat. To have a high proportion of meat in the carcass and good technological and nutritive indicators of meat, a necessary choice of such genetic potential of pigs which will be a good basis for increased production of muscle tissue of satisfactory quality. The a better quality of meat can be reached using different methods of selection and breeding. In planning and implementing a model breeding choice of breed is a very important factor, because it breeding combines the traits of pure breeds that are previous selection a profiled, with the aim of improving those traits which can be applied to repair breeding the plan.

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Kvalitet trupa i mesa tovljenika očeve rase landras i jorkšir

V. Vidović, A. Stoisavljević, D. Lukač, M. Stupar

Rezime

Cilj ovog istraživanja je bio da se utvrdi uticaj genotipa oca na kvalitet trupa i mesa. Istraživanje je urađeno na 18 hibridnih tovljenika dobijeni parnjem F_1 krmača sa po tri nerasta rase landras i jorkšir. Svaki otac je imao po tri potomka različite telesne mase, od 100 do 125 kg. Prosečna mesnatost polutki tovljenika očeve rase jorkšir je bila 56,67%, dok je kod tovljenika očeve rase landras bila 55,79%. Između potomaka očeve rase jorkšir i landras, nisu ustanovljene značajnije razlike u udelu pojedinih delova tela u trupu, s obzirom da se radi o dvema plodnim rasama koje imaju iste kriterijume selekcije. Analizom varijanse je takođe utvrđeno, da rasa nije uticala na udele pojedinih delova u polutkama. Između tovljenika očeve rasa jorkšir i landras je utvrđeno da nema statistički značajne razlike ($p > 0,05$) u tehnološkim i nutritivnim pokazateljima kvaliteta polutke, kao i da rasa oca nije uticala na tehnološki i nutritivni kvalietet mesa. Procenat proteina u mesu kao jednog od najvažnijih pokazatelja kvaliteta mesa, kod tovljenika obe rase se kretao oko 20 % . Kod svih žrtvovanih tovljenika, pH mišića se kretala u granicama normale od 5,4 do 5,8., tj. imali smo "normalno meso" bez pojave BMV i TČS mesa.

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STUDY ON FECUNDITY OF HYBRID SOWS

N. Metodiev[1], S. Gochev[2], M. Ignatova[1]

[1]Institute of Animal Science - Kostinbord
[2] Pig Company Ltd, Radko Dimitrievo, Shumen district
Corresponding author: n_metodiev@abv.bg
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Abstract: The aim of the study was to establish the effect of the parity of the PIC sows on the following indices: total litter size, size of live born, size of dead born piglets and size of mummified fetuses. 2090 farrows were studied, as they varied between 1st to 9th (n = I - 479 , II – 424, III - 334, IV - 369, V -217, VI - 152, VII - 112, VIII – 72 и XI – 31). The estimation of the parity was done by One –way ANOVA through statistical program SPSS 13.0. The significance of the studied factor was determined by values of F – criteria. When the factor influenced significantly, Post Hoc Test and LSD analysis were done to determine the significance of the differences between parities by t – test of Student. The obtained values of F-criteria showed, that the consecutiveness of parity influenced total litter size (F = (9.96, P<0.001), size of live born (F = 4.47 P<0.001), size of dead born (F = 29.07, P<0.001) piglets and mummified fetuses (F = 3.36, P<0.001). The highest total litter size had V – and VI- parity (15.16 and 15.20 respectively). The great number of live born piglets had parity V – at the average of 13.58 piglets, as the similar number had parities III and VI 13.51 and 13.56 respectively). From the obtained results about effect of parity, we could conclude that the effective reproductive use of hybrid PIC sows is up to parity VI inclusive.

Key words*:* sow, PIC, parity, litter size

Introduction

Sow prolificacy is a critical factor affecting the profitability of the swine industry (*Jiang and al., 2002)*. Prolificacy traits and longevity play an important role in efficient piglet production (*Serenius and Stalder, 2004*). High numbers of total and especially live-born piglets are the basic requirements for an effective piglet production (*Fischer et al., 2009*).

The objective of modern pig breeding is to exhaust the genetic potential in reproduction performance of sows regarding to litter size and number of weaned piglets per litter (*Wähner and Brüssow, 2009*). Litter size at birth is determined by

ovulation rate, fertilization rate, embryo and foetal survival (*Borges et al., 2005*). Foetal losses (mummified foetuses and stillborns) can vary from 5 to 15% (*Van der Lende, 2000*). Factors that influenced fetal losses are many and one of them is parity.

During last years, in Bulgaria is observed a large - scale import of highly-productive parent forms from world famous pig breeding companies – PIC – England, DanBred – Denmark, Topigs – Holand and etc., (*Stoikov and Katsarov, 2010*). These new trends in pig breeding in Bulgaria provoked us to do present study.

The aim of the study was to establish the effect of the parity of the PIC sows on the following indices: total litter size, size of live born, size of dead born piglets (stillbirths) and size of mummified fetuses.

Material and Methods

The study was carried out with hybrid sows PIC, raised in reproductive farm, owned of "Pig Company" Ltd, village Radko Dimitrievo, Shumen district. The 2090 farrows were studied, as they varied between 1st to 9th (n = I - 479 , II – 424, III - 334, IV - 369, V -217, VI - 152, VII - 112, VIII – 72 и XI – 31). All saws were artificially inseminated. The feeding was automatically. The weaning of pigs was at the age of 28-30 days. After weaning of pigs, sows were raised in individual cage until 35 day of new fertilization. After that they were united in groups (27 sows in group) until farrowing. The gilts were also raised in groups. It was done a strict control on fertilities and farrows in the farm.

The following indices were studied: total litter size, size of live born, difference between total litter size / live born piglets, size of dead born piglets (stillbirths) and size of mummified fetuses. The estimation of the parity was done by One –way ANOVA through statistical program SPSS 13.0. The significance of studied factor was determined by values of F – criteria. When the factor influenced significantly, Post Hoc Test and LSD analysis were done to determine the significance of the differences between parities by t – test of Student.

Results and Discussion

The obtained values of F-criteria (Table 1) for all parameters showed, that the parity influenced significantly ($P< 0.001$) total litter size, size of live born, stillbirths and size of mummified fetuses.

Total litter size was lowest et II parity – 13.37 and reached its maximum at V and VI parity – 15.16 and 15.2 respectively (Table 2). The differences between parities were presented at Table 3. Parities I and II significantly differed to parities

III, IV, V and VI. These results were in correspondence to postulate that the litter size increases from first to third parity and holds high to sixth parity and after that goes down (*Stoikov and Katsarov, 2010*).

Table 1. Values of F-criteria for the effect of *parity* on the indices: total litter size, size of live born, stillbirths and size of mummified fetuses.

Index	*Source of Variation*	DF	F	Significance
Total litter size	Between Groups	8	9.961	***
	Within Groups	2081		
	Total	2089		
Live born piglets	Between Groups	8	4.47	***
	Within Groups	2081		
	Total	2089		
Stillbirths	Between Groups	8	29.07	***
	Within Groups	2081		
	Total	2089		
Mummified fetuses	Between Groups	8	3.86	***
	Within Groups	2081		
	Total	2089		

- Note. *** **Significant differences at P< 0.001**

The lowest values of parameters live born pigs had parity VIII (Table 2). Parities II, VII and IX had also low values (Table 2). The difference between parities was presented at Table 4. Parity II was significantly the lowest in comparison to parities I, III, IV, V and VI.

It is interesting to analyze the next parameter, called reproductive efficiency which mean the differences between total litter size and live born pigs. The smallest difference between those parameters had parities I and II, and after that the difference increased, as reached its maximum at parities VII and IX (Table 2). The number of live born piglets determines the number of weaned pigs, so this parameter has big economic importance (*Stoikov and Katsarov, 2010*).

Table 2. Mean value of the studied indices in each parity

Parity	Number of farrows	Total litter size, $\bar{X} \pm SE$	Live born piglets, $\bar{X} \pm SE$	Difference between total litter size / live born piglets	Stillbirths, $\bar{X} \pm SE$	Mummified fetuses, $\bar{X} \pm SE$
I	479	13.76 ± 0.14	13.25 ± 0,13	0.51	0.44 ± 0.4	0.07 ± 0.02
II	424	13.37 ± 0.18	12.70 ± 0.17	0.67	0.61 ± 0.05	0.07 ± 0.01
III	334	14.32 ± 0.18	13.51 ± 0.18	0.81	0.64 ± 0.05	0.18 ± 0.04
IV	269	14.71 ± 0.21	13.38 ± 0.20	1.33	1.17 ± 0.10	0.14 ± 0.04
V	217	15.16 ± 0.23	13.58 ± 0.22	1.58	1.40 ± 0.13	0.18 ± 0.03
VI	152	15.20 ± 0.25	13.56 ± 0.25	1.64	1.48 ± 0.13	0.16 ± 0.04
VII	112	14.44 ± 0.31	12.44 ± 0.26	2.0	1.75 ± 0.18	0.24 ± 0.05
VIII	72	13.76 ± 0.35	12.11 ± 0.32	1.65	1.33 ± 0.14	0.32 ± 0.10
IX	31	14.81 ± 0.41	12.71 ± 0.37	2.1	1.90 ± 0.25	0.19 ± 0.08

Table 3. Significance of the differences between parities, concerning total liter size

Parity	I	II	III	IV	V	VI	VII	VIII	IX
I	-	NS	*	***	***	***	*	NS	NS
II	NS	-	***	***	***	***	**	NS	*
III	*	***	-	NS	**	**	NS	NS	NS
IV	***	***	NS	-	NS	NS	NS	*	NS
V	***	***	**	NS	-	NS	NS	**	NS
VI	***	***	**	NS	NS	-	NS	**	NS
VII	*	**	NS	NS	NS	NS	-	NS	NS
VIII	NS	NS	NS	*	**	**	NS	-	NS

- Note. Significant differences at , * P< 0.05; ** P< 0.01; ***P< 0.001

Table 4. Significance of the differences between parities, concerning live born piglets

Parity	I	II	III	IV	V	VI	VII	VIII	IX
I	-	**	NS	NS	NS	NS	**	**	NS
II	**	-	***	**	***	**	NS	NS	NS
III	NS	***	-	NS	NS	NS	**	***	NS
IV	NS	**	NS	-	NS	NS	**	**	NS
V	NS	***	NS	NS	-	NS	**	***	NS
VI	NS	**	NS	NS	NS	-	**	***	NS
VII	NS	NS	**	**	**	**	-	NS	NS
VIII	**	NS	***	**	***	***	NS	-	NS

- Note. Significant differences at , * P< 0.05; ** P< 0.01; ***P< 0.001

With increasing of the parity, stillbirths also increased (Table 2), as parity I had lowest stillbirths – 0.61. This conclusion corresponded to findings of other researchers - Leenhouwers et al., 1999; Le Cozler et al., 2002; Canario et al., 2006. Vanderhaeghe et al. (2010, who said that with increasing of parity also stillbirths increased. Borges et al., 2005, observed that parity >5 and parity 1 sows had higher

and lower odds of stillbirth, respectively, in comparison to parity 2–5 sows. The authors studied four commercial farms in Brazil as two farms were with sows PIC Camborough with parities from one to nine. The association between higher parities and stillbirth risk could be attributed to a poor uterine muscle tone leading to less efficient expulsion and prolonged farrowing (*Pejsak, 1984*).

Table 5. Significance of the differences between parities, concerning stillbirths

Parity	I	II	III	IV	V	VI	VII	VIII	IX
I	-	*	*	***	***	***	***	***	***
II	*	-	NS	***	***	***	***	***	***
III	*	NS	-	***	***	***	***	***	***
IV	***	***	***	-	NS	*	***	NS	**
V	***	***	***	NS	-	NS	*	NS	*
VI	***	***	***	*	NS	-	NS	NS	NS
VII	***	***	***	***	*	NS	-	*	NS
VIII	***	***	***	NS	NS	NS	*	-	*

- Note. Significant differences at , * P< 0.05; ** P< 0.01; ***P< 0.001

The same is situation about mummifications - with increasing the parity, mummified fetuses also increased (Table 2). The differences between parities about stillbirths and mummified fetuses were presented at Tables 5 and 6 respectively. Borges et al., 2005, reported that sows of parity 1 and >5 had higher odds (OR = 3.5 and 2.0, respectively) of mummified foetuses compared to parity 2–5. In our study the lowest values of mummified fetus had parity I and II – 0.07 (table 2). It is well known, that there are two main causes of mummies - first is because of the large litter size and insufficient space in the uterus and second - infectious disease. Younger sows could be at a risk of mummification due to a likely lower level of immunity provided by an insufficient time of exposure to endemic pathogens (Dial et al., 1992). In our case the lowest numbers of mummies were at parities I and II, so we suppose that larger litter was the main cause for mummifications.

Table 6. Significance of the differences between parities, concerning mummified fetuses

Parity	I	II	III	IV	V	VI	VII	VIII	IX
I	-	NS	*	*	*	NS	**	***	NS
II	NS	-	**	*	*	*	**	***	NS
III	*	**	-	NS	NS	NS	NS	*	NS
IV	*	*	NS	-	NS	NS	NS	*	NS
V	*	*	NS	NS	-	NS	NS	*	NS
VI	NS	*	NS	NS	NS	-	NS	*	NS
VII	**	**	NS	NS	NS	NS	-	NS	NS
VIII	***	***	*	*	*	*	NS	-	NS

- Note. Significant differences at , * P< 0.05; ** P< 0.01; ***P< 0.001

Conclusions

The consecutiveness of parity influenced all studied reproductive parameters: total litter size ($F = 9.96$, $P<0.001$), size of live born ($F = 4.47$ $P<0.001$), size of dead born ($F = 29.07$, $P<0.001$) piglets and mummified fetuses ($F = 3.36$, $P<0.001$).

The great number of live born piglets had parity V – at the average of 13.58 piglets, as the similar number had parities III and VI (13.51 and 13.56 respectively).

With increasing the parity foetal losses also increased. The smallest difference between total litter size and number of live born piglets had parities I and II, and after that the difference increased, as reached its maximum at parities VII and IX.

From the obtained results about effect of parity, we could conclude that the effective reproductive use of hybrid PIC sows is up to parity VI inclusive.

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Studija o plodnosti hibridnih krmača

N. Metodiev, S. Gochev, M. Ignatova

Rezime

Cilj istraživanja bio je da se utvrdi efekat pariteta PIC krmača na osnovu sledećih indikatora: ukupna veličina legla, broj živirođenih, mrtvorođenih prasadi i broj mumificiranih fetusa. Ispitano je 2090 prašenja, od 1. do 9. (n = I - 479, II - 424, III - 334, IV - 369, V -217, VI - 152, VII - 112, VIII - 72 i IX - 31). Procena pariteta urađena je u jednosmernom ANOVA statističkom programu SPSS 13.0. Značaj ispitivanih faktora određen je vrednostima f - kriterijuma. Kada je faktor uticao značajno, Post hoc i LSD analize su urađene da se utvrdi značaj razlika između pariteta korišćenjem Student T - testa.

Dobijene vrednosti F-kriterijuma su pokazale, da uzastopnost pariteta utiče na ukupnu veličinu legla (F = (9.96, p <0.001), broj živorođenih (F = 4,47 p <0,001), broj mrtvorođenih (F = 29,07 ., p <0.001) prasadi i mumificiranih fetusa (F = 3.36, p <0.001). Najveću ukupnu veličinu legla imao je V - i VI -paritet (15.16 i 15.20, respektivno) Veliki broj živorođene prasadi utvrđen je za paritet V - u proseku 13.58 prasadi, dok je slična vrednost utvrđena I za paritete III i VI 13.51 i 13.56, respektivno. Iz dobijenih rezultata o uticaju pariteta, možemo zaključiti da je efikasno reproduktivno korišćenje hibridnoih PIC krmača zaključno sa paritetom VI.

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EFFECT OF FEEDING RAPESEED MEAL TO WEANED PIGS ON PERFORMANCE AND TOTAL SERUM CHOLESTEROL CONCENTRATIONS

M. Todorova, M. Ignatova

Institute of Animal Science, Kostinbrod, Bulgaria
Corresponding author: mariq_todorova_1@abv.bg
Original scientific paper

Abstract: The experiment was conducted to evaluate the effect of use of rapeseed meal instead of soybean meal in weaned pigs diet on performance and total serum cholesterol concentration. Thirty weaned pigs cross-breed Youna (initial body weight 14.06 ± 0.24 kg) with two replicates were randomly allocated to two experimental groups for six weeks. The experimental design consisted of two dietary treatments. The diet used as control was standard compound feed based on corn and soybean meal. The experimental diet contained 7.5 % rapeseed meal. The diets were formulated to be isoenergetic and isonitrogenous. During the experiment pigs had ad libitum access to feed and water. Individual pigs body weights were measured initially and on day 14, 28, and 42 of experiment. Feed intake and feed conversion ratio of the subgroups were determined every 14th day. At the end of the experiment blood samples were collected from the pigs to determine total serum cholesterol concentrations. Inclusion of 7.5 % rapeseed meal in the diet of weaned pigs tended to have negative effect on the performance. Average daily gain was decreased by 5.7%. Average daily feed intake was increase by 3.4 %. Feed conversion ratio was worsening by 20.6 %. Feeding rapeseed meal decreased total serum cholesterol concentration by 5.1 %.

Key words: rapeseed meal, weaned pigs, performance, total serum cholesterol concentration.

Introduction

The expansion of biofuel industry will result in a significant increase in volume of rapeseed meal. Rapeseed meal can be used as feedstuff in livestock industry or can be used as soil amendment or fertilizer *(Bonnardeaux., 2007)*.

As feed ingredient rapeseed meal has very good nutritional properties. After oil extraction, rapeseed meal has high content of crude protein (35 - 40 %) and higher neutral detergent fiber (NDF) content then soybean meal because hulls

are not eliminated and represent about 30 % of the meal which reduced digestible energy content respect to soybean meal (2.6 vs. 3.4 Mcal/kg) *(Mariscal – Landin et al., 2008)*. The problem with high fiber content can be solved by dehulling *(Lange et al., 1998)*. According to oil extraction methods the residual oil content in meal ranged between less than 2 % and 15 % *(Canola meal industry guide, 2009 4th Ed; Simpson., 2010)*. The use of rapeseed meal in swine nutrition is limited by the antinutritional factors – glucosinolates, sinapine and tannins.

Maximum inclusion level of rapeseed meal in weaned pig diet is 5 % *(Canola meal industry guide, 2009 4th Ed; Simpson, 2010)*. The investigations show that rapeseed meal in weaned pig diet reduced performance *(David Ekpe et al., 2001)* or did not influence performance *(Aherne and Baidoo, 1991)*.

In experiments with humans has been proven that consumption of canola oil lowered total serum cholesterol concentration *(Lichtenstein et al., 1993)*.

The aim of this study was to investigate the effect of rapeseed meal in weaned pig diet on growth performance and total serum cholesterol concentration.

Material and Methods

Thirty weaned pigs crossbreed Youna were randomly allocated to two experimental groups with two replicates. Initial body weights of pigs were 14.06±0.24 kg. The pigs were housed in experimental base of the Institute of animal science – Kostinbrod. Experimental period was six weeks. Two dietary treatments were compared in this experiment. Standard compound feed based on corn and soybean meal was used as control diet. Experimental diet was formulated to contained 7.5 % rapeseed meal instead of corresponding amount of soybean meal. The rapeseed meal contain 96.41 % dry matter, 34.42 % crude protein, 2.76 % crude fat, 12.39 % crude fiber, 43.65 % ether extract and 6.78 % ash. The concentration of glucosinolates and erucic acid was 10.5 μmol/g and 0.06 % respectively. Control and experimental diets were formulated to be isonitrogenous and isoenergetic. Diet composition and nutrient content of diets were shown in table 1. Pigs were fed ad libitum and had free access to water by nipple watering trough.

During the experimental period were observed individual body weight of pigs and feed consumption in 14 day intervals. On the bases of these data were calculated average daily gain, average daily feed intake and feed conversion ratio.

At the end of the study blood samples were collected from pigs to determined total serum cholesterol concentration. Total serum cholesterol concentration was determined by the method of *Mrskos* and *Tovarek* as described by *Ibrishimov and Lalov (1987)*.

The research data analyses were made by the usual variation statistics methods and Student`s t – test was used to compared means.

Table 1. Diet composition and nutrient content of experimental diets.

Ingredient %	Control group	Experimental group
Corn	47.81	46.31
Wheat	21	21
Sunflower meal	4	4
Soybean meal	24	18
Rapeseed meal		7.5
Vitamin/trace mineral premix	0.20	0.20
Limestone	1.50	1.50
Dicalcium phosphate	0.92	0.92
Salt	0.20	0.20
Lysine	0.22	0.22
Methionine	0.10	0.10
M-tox	0.05	0.05
Nutrient content %		
Dry matter	91.61	91.67
Crude protein	20.18	19.23
Ether extract	3.13	2.83
Ash	5.63	5.05
Crude fiber	3.80	4.81
Calcium	0.98	0.81
Phosphorus	0.514	0.548

Result and Discussion

No significant differences were observed in explored parameters in this experiment. These results are in agreement with the results of *Aherne and Baidoo (1991)*. However there were numerical differences between the groups. Pigs fed rapeseed meal tended to have reduced performance.

Body weights of pigs are shown in table 2. Pigs from the experimental group had lower body weights compared to the control group. At the end of the experiment control group had 6 % higher body weight compared to the experimental group.

Table 2. Body weight of pigs, kg.

Period	Control group, x□±SD	Experimental group, x□±SD	%
At the beginning of the experiment	13.89±2.17	14.23±2.57	
At the end of 2nd week	20.86±3.74	20.75±4.3	99.5
At the end of 4th week	26.4±4.33	25.07±4.39	95
At the end of the experiment	35.13±5.61	33.02±5.35	94

Data characterizing average daily gain are shown in figure 1. Inclusion of 7.5 % rapeseed meal in diet depressed average daily gain in weaned pigs. This depression in average daily gain was by 6.4, 22.1 and 9 % at the end of the 2nd week, of the 4th week and at the end of the 6th week of the experiment, respectively. For the whole experiment average daily gain was depressed by 5.7 %. These results are in agreement with other research study *(David Ekpe et al., 2001).*

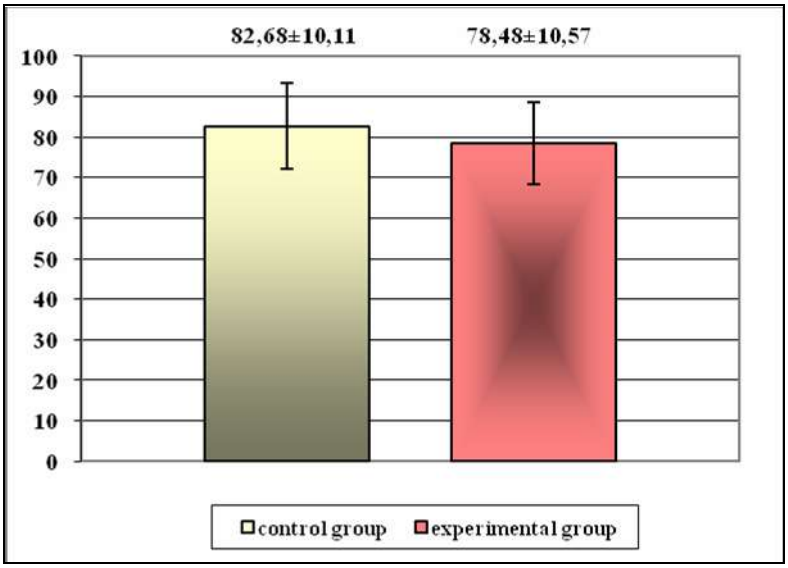

Figure 2. Total serum cholesterol of pigs, mol%.

Average daily feed intake was similar among treatment (table 3). Rapeseed meal did not reduced feed intake. We observed a slightly increase in average daily feed intake of experimental group by 3.4 % for all experimental period. Our results

are in contrast with the results of other studies that show decrease in average daily feed intake *(David Ekpe et al., 2001)*.

Table 3. Feed intake kg/day and feed conversion ratio kg/kg gain.

Indices	Control group	Experimental group	%
Average daily feed intake	1.043±0.18	1.078±0.17	103.4
Feed conversion ratio	2.043±0.26	2.463±0.52	120.6

Feed conversion ratio of the experimental group was worsening by 20 % compared to the control group. Data are shown in table 3.

Data about total serum cholesterol concentration are shown in figure 2. Pigs from the experimental group tended to have lowered total serum cholesterol concentration in experimental group by 5.1 %.

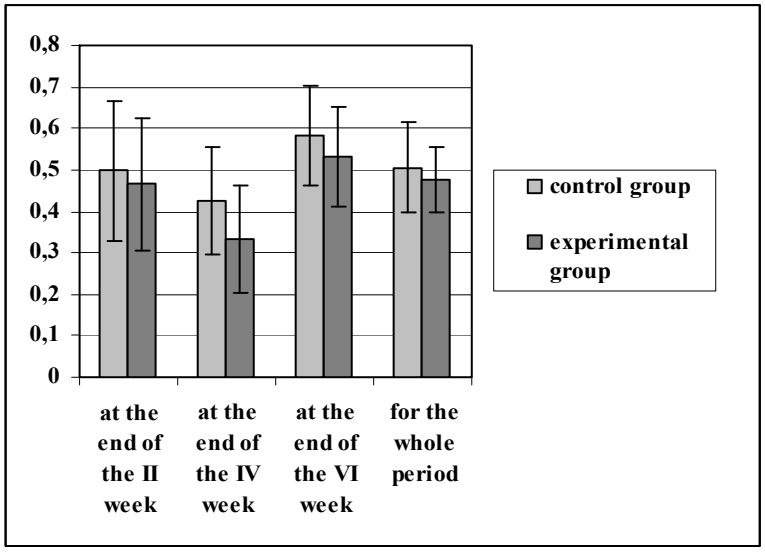

Figure 1. Average daily gain of pigs, kg

Conclusion

The results of this study have shown that inclusion of 7.5 % rapeseed meal instead of soybean meal in weaned pig diet tended to have negative effect on growth performance. At the end of the experimental period body weight of the experimental group was lowered by 6 %. For the whole experiment average daily gain was depressed by 5.7 %. Feed conversion ratio was worsening by 20 %. Rapeseed meal lowered total serum cholesterol concentration by 5.1 %.

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Uticaj korišćenja sačme uljane repice u ishrani odlučene prasadi na proizvodne rezultate i ukupni nivo koncentracije holesterola u krvi

M. Todorova, M. Ignatova

Rezime

Cilj ovog eksperimenta bio je da se ispita uticaj korišćenja obroka sa sačmom uljane repice umesto sojine sačme u ishrani odlučene prasadi na performanse i ukupni holesterol u serumu.

U eksperimentu se koristilo trideset odlučene prasadi meleza Youna (početne težine 14,06 ± 0,24 kg) nasumično podeljenih u dve eksperimentalne grupe sa dva ponavljanja. Eksperimentalni period je 42 dana. Kontrolna ishrane je bila sa smešom na bazi kukuruza i sojine sačme. Eksperimentalni obrok sadrži 7.5% sačme uljane repice. Ishrana je formulisana kao izoenergetska i izo-azotna. U toku ogleda svinje su imale *ad libitum* pristup hrani i vodi. Merena je telesna masa pojedinačnih svinja na početku i 14, 28, i 42. dan eksperimenta. Unos hrane i konverzija hrane podgrupa utvrđene su svakog 14. dana. Na kraju eksperimenta uzorci krvi su uzimani od svinja za određivanje ukupne koncentracije holesterola u serumu.

Uključivanje sačme uljane repice u ishrani ima tendenciju smanjenja performansi rasta svinja. Na kraju eksperimenta, eksperimentalna grupa je imala za 6% manju telesnu masu u odnosu na kontrolnu grupu. Prosečan dnevni prirast je smanjen za 6,4; 22,1 i 9% na kraju 2. nedelje, 4. nedelje i na kraju 6. nedelje eksperimenta, respektivno. Za ceo eksperiment prosečan dnevni prirast je niži za 5,7%. Konverzija hrane u eksperimentalnoj grupi je lošija za 20% u odnosu na kontrolnu grupu, ali smo primetili malo povećanje prosečnog dnevnog unosa hrane ogledne grupe za 3,4% za sve eksperimentalne periode.

Ukupni holesterola u serumu eksperimentalne grupe je smanjen za 5,1%.

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CHEMICAL AND MICROBIOLOGICAL ANALYSIS OF FRESH, FROZEN AND EMULSIONS OF PORK SKINS

D. Andronikov[1], D.Naseva[1], N. Taskov[2], D. Saneva[2], A. Kuzelov[1]

[1]Department of Food Technology and processing of animal products, Faculty of Agriculture, University Goce Delcev Stip, Krste Misirkov bb, Stip, R. Macedonia
[2] Faculty of Tourism and Business Logistics, University Goce Delcev Stip, Krste Misirkov bb, Stip, R. Macedonia
Corresponding author: darko.andronikov@ugd.edu.mk; tel: +389 32 550 111.
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Abstract: The objective of our survey was to determine the content of proteins in fresh and frozen pork skins, as well as in the emulsion of skins and the influence of different acids on their microbiology before and after the cutting. Altogether 120 kg pork skins (selected by random choice) split in 2 groups per 60 kg fresh and 60 kg frozen skins and immersed in three different combinations of acid solutions (acetic, lactic, wine, lemon) and water. 6 groups have been obtained for further analysis: fresh and frozen skins immersed in A, B, C solutions. Before and after the immersing, samples were taken from all groups for chemical and microbiological analysis. Previously grinded and well homogenized, the following parameters were analyzed: water, fats, ash and proteins. Pork skins were immersed for 72 in total in a solution, then washed with water and well drained. The manner of preparation was; 20 kg skins + 20 kg water (ice) + 200 g soy proteins. The so prepared emulsion of skins undergoes chemical and microbiological analysis. The content of proteins in emulsion of fresh skin is bigger when compared to frozen pork skins. The microbiological analysis showed us that in pork skins immersed in different solutions of acids and water, as well as in the emulsion of skins, no bacteria were found of the following types *clostridium, staphylococcus, proteus,* escherichia, and the total number of bacteria (bacillus) showed decrease of bacteria in all groups of fresh and frozen pork skins. The biggest decrease in the total number of bacteria before and after the immersed skins, as well as on the emulsion of skins, was found in the A solution.

Key words: Pork skins, protein, emulsion of skins, chemical and microbiological analysis.

Introduction

The skin of pigs is in direct contact with polluting sources and represents excellent place for developing numerous surface microbiological pollutions *(Kim et al., 199*6). The pork skin represents a great source of proteins, and the emulsion of skins (water, fats, proteins) serves as a supplement to many boiled products *(Osburn and Mandigo 1998; Radetic 2000)*. Due to the joint tissues (collagen, elastin, retikulin) and the proteins are very hard and that is why they are treated by means of different combinations of acids (lactic, acetic, wine, lemon) and water for the purpose of softening *(Andronikov et al. 2013)*. At the same time, the immersing in such solutions serves as a good bacteriological protection. Different types of bacteria (*clostridium, staphylococcus, proteus, escherichia, salmonella, listeria*) were tested on fresh pork and chicken skins by many authors *(Pipek et al. 2006; Trivedi et al. 2008; Lecompte et al. 2009; Rikke et al. 2011; Chaine et al. 2013)*.
The objective of this survey was to determine the content of proteins and fats in fresh and frozen skins, as well as in the emulsion of skins before and after the immersing in different solutions of acids and water. Moreover, the aim was to witness the microbiological difference between fresh and frozen pork skins before and after the immersing in different solutions of acids and water and the microbiological difference between the emulsion of fresh and frozen pork skins.

Material and Methods

A total of 120 kg pork skins were taken (selected randomly), divided in two groups - 60 kg fresh and 60 kg frozen, immersed in three different combinations of acid solution (acetic, lactatic, tartaric, citric) and water. Six groups were obtained - fresh (3 x 20 kg) and frozen (3 x 20 kg) skins immersed in:

- A - solution (15 g acetic acid, 200 g lactatic acid, 100 g tartaric acid, 100 g citric acid, 18,5 l water),
- B - solution (200 g lactatic acid, 100 g tartaric acid, 19,7 l water),
- C - solution (50 g acetic acid, 200 g lactatic acid, 19,7 l water).

Pork skins are separated from the bacon using a special machine called DERINDER. This machine is composed of a base, electric motor, grinning roller and sharp knife. The bacon and the skin are putted on the drum DERINDER - with the skin downward, the roller takes the pieces and move them right to the blade which peels off the skin of bacon. Samples from all groups were taken before and after immersion, for chemical and microbiological analysis. Earlier samples were cutted ground and well homogenized in a small mixer for home use. Pork skins

were putted in solution for 72 hours, then washed and well drained. Then every group of skins (6 x 20 kg) is separately placed in a cutter and mixed with 20 kg water + 200 g soy proteins, whereby 6 groups of skin emulsions are prepared. Chemical and bacteriological analyses have been done on all groups.

Chemical analyses

Total nitrogen (TN) was determined according to the Kjeldahl method. Moisture content was determined by drying at (103 ± 2) °C to constant mass. The intramuscular fat content was determined according to AOAC International method, with petroleum ether as solvent. Minerals were determined by burning and combustion (4 – 5 h) at 525 – 550 °C.

Microbial analysis

Following bacteria were determined: Total bacteria (*Bacillus*) number - ISO 4833 / 2003. Have sown on nutrient agar to 37 °C during 24 hours. *Staphylococcus* ISO 6888 - 1 / 1999. Have sown on ETGP agar (barit parker agar) after termostating on 37 °C during 24 hours. *Enterobacteriaceae* ISO 21528 - 1 / 2004; ISO 21528 - 2 / 2009. *Escherichia coli* are sown on lactoza bujon and brilian green, thermostated on 37 °C during 24 - 48 hours. *Clostridium* sown on sulfiten agar, thermostated on 37 °C during 24 - 48 hours

Data were transformed into $\log_{10}$ CFU/g before comparison of means.
The results were statistically processed using mathematical program Microsoft EXEL ANOVA (single factor) 2009 / 2013.

Results and Discussion

Chemical composition

The results of the chemical analysis of fresh skin before and after the immersing in solutions of acids and water are presented in Table No. 1. In fresh skin before immersing in solution, we established significant differences ($p < 0.05$) in the content of water. After immersing the frozen skins, a significant difference was noticed ($p < 0.05$) in the content of water and fats between Frozen skin+ A sol. and Frozen skin+ C sol. The content of proteins at frozen skin has also shown significant difference ($p < 0.05$) between Frozen skin+ B sol. and Frozen skin+ C sol. Our results were similar to the results obtained by *Radetic 2000; Andronikov et al. 2013*.

Table 1. Results of chemical composition of fresh and frozen pork skins before and after immersion in different acids solutions.

Before immersion			
Parameters	Fresh skin $\overline{X}$ ± Sd	Fresh skin $\overline{X}$ ± Sd	Fresh skin $\overline{X}$ ± Sd
Water	57.08 ± 0.28A	58.85 ± 1.37	52.36 ± 1.67C
Fats	29.06 ± 1.04	27.90 ± 1.27	35.77 ± 0.48
Proteins	13.10 ± 0.16	12.90 ± 0.10	11.52 ± 0.70
Minerals	0.26 ± 0.04	0.29 ± 0.05	0.47 ± 0.02
After immersion			
Parameters	Fresh skin + A sol. $\overline{X}$ ± Sd	Fresh skin + B sol. $\overline{X}$ ± Sd	Fresh skin + C sol. $\overline{X}$ ± Sd
Water	61.04 ± 1.15	56.28 ± 0.31	54.59 ± 0.79
Fats	24.27 ± 1.43	28.96 ± 0.22	34.94 ± 0.20
Proteins	12.62 ± 0.38	14.35 ± 0.06	11.51 ± 0.09
Minerals	0.27 ± 0.02	0.19 ± 0.01	0.53 ± 0.39
Before immersion			
Parameters	Frozen skin $\overline{X}$ ± Sd	Frozen skin $\overline{X}$ ± Sd	Frozen skin $\overline{X}$ ± Sd
Water	57.66 ± 0.31	52.40 ± 0.49	54.60 ± 0.88
Fats	28.99 ± 0.15	35.92 ± 0.95	31.56 ± 0.6
Proteins	13.17 ± 0.07	11.25 ± 0.65	12.76 ± 0.13
Minerals	0.23 ± 0.03	0.19 ± 0.01	0.53 ± 0.39
After immersion			
Parameters	Frozen skin+ A sol. $\overline{X}$ ± Sd	Frozen skin+ B sol. $\overline{X}$ ± Sd	Frozen skin+ C sol. $\overline{X}$ ± Sd
Water	63.44 ± 1.63A	63.82 ± 0.38	59.58 ± 0.27C
Fats	24.22 ± 1.50A	34.47 ± 0.49	31.95 ± 0.51C
Proteins	12.95 ± 0.04	11.49 ± 0.05B	13.18 ± 0.31C
Minerals	0.26 ± 0.02	0.21 ± 0.04	0.29 ± 0.03

$\overline{X}$ = mean, Sd = standard deviation; mean values with different capital letters (A, B, C) between each row differ significantly ($p < 0.05$).

The chemical composition of fresh and frozen skins emulsion is presented in Table 2.

The differences between the different parameters are not significantly important ($p < 0.05$). In general, the results as regards the content of proteins and water at fresh skin are bigger than at frozen skin. This means the fresh skin when compared to frozen skin better joints the added proteins and water.

Table 2. Results of chemical composition of emulsion of fresh and frozen pork skins in different acids solutions.

Fresh skin			
Parameters	Emulsion of skin + A sol. $\overline{X}$ ± Sd	Emulsion of skin + B sol. $\overline{X}$ ± Sd	Emulsion of skin + C sol. $\overline{X}$ ± Sd
Water	63.40 ± 1.1	58.50 ± 1.13	56.20 ± 0.54
Fats	23.18 ± 1.49	25.80 ± 0.52	31.28 ± 0.65
Proteins	13.17 ± 0.02	15.50 ± 0.45	12.10 ± 0.72
Minerals	0.25 ± 0.03	0.20 ± 0.28	0.44 ± 0.45
Frozen skin			
Parameters	Emulsion of skin + A sol. $\overline{X}$ ± Sd	Emulsion of skin + B sol. $\overline{X}$ ± Sd	Emulsion of skin + C sol. $\overline{X}$ ± Sd
Water	66.50 ± 0.25	55.05 ± 0.42	55.50 ± 0.22
Fats	21.15± 0.52	33.50 ± 0.48	32.30 ± 0.35
Proteins	12.12 ± 0.48	11.78 ± 0.32	12.05 ± 0.48
Minerals	0.28 ± 0.22	0.20 ± 0.55	0.15 ± 0.25

$\overline{X}$ = mean, Sd = standard deviation;

Microbiological analysis

Table 3. Results of microbiological analysis of fresh and frozen pork skins before and after immersion in different solutions of acids and water.

Bacteriology **Before immersion**	Bacteriology **Before immersion**	Bacteriology **Before immersion**
Fresh skin A Total bacteria number	Fresh skin B Total bacteria number	Fresh skin C Total bacteria number
2.50 log CFU/g	2.57 log CFU/g	2.65 log CFU/g
Frozen skin A Total bacteria number	Frozen skin B Total bacteria number	Frozen skin C Total bacteria number
2.44 log CFU/g	2.47 log CFU/g	2.54 log CFU/g
Bacteriology **After immersion**	Bacteriology **After immersion**	Bacteriology **After immersion**
Fresh skin + A sol. Total bacteria number	Fresh skin + B sol. Total bacteria number	Fresh skin + C sol. Total bacteria number
/	1.30 log CFU/g	1.69 log CFU/g
Frozen skin + A sol. Total bacteria number	Frozen skin + B sol. Total bacteria number	Frozen skin + C sol. Total bacteria number
/	1.30 log CFU/g	1.84 log CFU/g

The microbiological analysis results of the fresh and frozen pork skins before and after immersion in different solutions of acids and water are given in Table No. 3. We found no statistical significant difference ($p < 0.05$) between the fresh and frozen skin. It is obvious that different solutions of acids and water affected the decrease of the total number of bacteria (bacillus) of fresh and frozen skin. This is especially noted with solutions A and B, but those differences are not statistically significant ($p > 0.05$).

The microbiological analysis results of emulsion of fresh and frozen pork skins in different acid solutions are given in Table No. 4. The total number of bacteria in the process of cutting is increased in all groups of emulsion of fresh and frozen skin in A, B, C solution. The results obtained for the emulsion of fresh skin in all solutions of acids and water are lower than the emulsion of frozen skin. The results obtained had no statistical significant differences ($p > 0.05$).

Table 4. Results of microbiological analysis of emulsion of fresh and frozen pork skins in different acids solutions.

Bacteriology	Bacteriology	Bacteriology
Emulsion of fresh skin + A sol. Total bacteria number	Emulsion of fresh skin + B sol. Total bacteria number	Emulsion of fresh skin + C sol. Total bacteria number
2.74 log CFU/g	2.85 log CFU/g	2.87 log CFU/g
Emulsion of frozen skin + A sol. Total bacteria number	Emulsion of frozen skin + B sol. Total bacteria number	Emulsion of frozen skin + C sol. Total bacteria number
2.85 log CFU/g	2.87 log CFU/g	2.90 log CFU/g

Conclusion

Our survey has shown us the current chemical composition, including the content of proteins, water and fats in fresh and frozen pork skins on one part of the territory of the Republic of Macedonia. We found that in the emulsion of skins, fresh pork skins joint better proteins and water. In pork skins immersed in different solutions of acids and water, as well as in the emulsion of skins, no bacteria were found of the types *clostridium, staphylococcus, proteus, escherichia*, and the total number of bacteria (bacillus) showed decrease of bacteria in all groups of fresh and frozen pork skins. The best results and the biggest decrease in the total number of

bacteria before and after the immersion of skins, as well as the emulsion of skins, were noted in solution A.

Hemiska i mikrobiološka analiza svežih, zamrznutih i emulzija svinjskih kožica

D. Andronikov, D.Naseva, N. Taskov, D. Saneva, A. Kuzelov

Rezime

Cilj našeg istraživanja bio je da se utvrdi sadržaj proteina u sveže i zamrznute svinjske kože i kože od emulzije kao i uticaj različitih kiselina na njihovu mikrobiologiju pre i posle kuterovanja. Uzeta su ukupno 120 кg svinjskih kožica (odabrani su slučajnim izborom) podeljene u 2 grupe po 60 кg svežih i 60 кg zamrznutih kožica i potopljene u tri različite kombinacije rastvora kiselina (sirћetna, mlečna, vinska, limunska) i vode. Dobijene su 6 grupe za dalju analizu: sveže i zamrznute kože potopljene u A, B, C rastvora. Pre i nakon potapljanja su uzorci uzeti iz svih grupa za hemiske i mikrobiološke analize. Ranije samljeveni i dobro homogenizovani, analizirani su sledeће hemiske paramtre: voda, pepel, masti i proteina. Svinske kožice su bile potopljene 72 sata u rastvoru, a zatim su oprani vodom i dobro su iscedene. Tako pripremljene stavljene su u kuter u odnosu 20 кg kožica + 20 кg vode (leda) + 200 g sojinih proteina. Na ovaj način je pripremljena emulzija od kože a potom i izvrsena je hemiska i mikrobiloška analiza. Sadržaj proteina u emulziju od svežih kožica je veћi od emulzije zamrznute svinjske kože. Mikrobiloška analiza nam je pokazala da u svinjskih kožica potopljene u različitim rastvorima kiselina i vode kao i u emulzijom od kože nisu pronaћeni bakterije iz vrste *clostridium, staphylococcus, proteus, escherichia*, a ukupan broj bakterija (bacila) pokazao je smanjenje bakterija u svim grupama svežih i zamrznutih svinjskih kožica. Najveће smanjenje u ukupnom broju bakterija pre i posle potapljanja kože i emulzije od kože utvrћeno je u rastvoru A.

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IMPACT OF FUNCTIONAL MIX AND STARTER CULTURES ON THE SENSORY PROPERTIES OF PERMANENT SAUSAGES PRODUCED IN INDUSTRIAL CONDITIONS

A. Kuzelov[1], D. Naseva,[1] N. Taskov,[2] D. Saneva,[2] D. Spasova,[1] D. Andronkov[1]

[1]Department of Food Technology and processing of animal products, Faculty of Agriculture, University Goce Delcev Stip, Krste Misirkov bb, Stip, R. Macedonia
[2]Faculty of Tourism and Business Logistics, University Goce Delcev Stip, Krste Misirkov bb, Stip, R. Macedonia
Corresponding author: aco.kuzelov@ugd.edu.mk
Original scientific paper

Abstract: The aim of their search is the impact little bit of functional mixed (composed of glukono delta lactate, ascorbic acid and vitamin C) and starter culture (commercial preparation F - SC111 Bactoferm product company Chr Hansen DK). This product contains a mixed culture composed of *Lactobacilus sakei* and *Staphyococus Camosus* frozen in dry form. In three varieties of tea sausage: I - added a simple sugar sucrose which serve as a control sample, II - with the addition of starter culture, III - functional mix of fermented sausages. The best results in terms of sensory properties rehearsal was produced with the addition of starter culture and lowest sample with added sucrose.

Keywords: functional mix, starter culture, sucrose.

Introduction

The most numerous group of meat is sausages. We have several hundred species with several hundred thousand commercial names. In Europe the production of sausages began about 250 years ago in Italy. Italian masters 150 years ago this technique was transferred to Hungary from where it began to spread throughout Europe. The production of fermented sausages in R. Macedonia is the beginning of 90 years of last century. The first attempts were made to produce tea sausage. Until then in R. Macedonia fermented products are supplied from other former Yugoslav republics. Fermented sausages are demanded in the market due to its culinary and nutritional properties. The quality of the raw material, the long production process, which requires consumption of large amounts of energy makes

the production of these sausages expensive *(Vesković - Moračanin and Obradović 2009).*

Today fermented sausages produced in industrial conditions during the process of maturation takes place in special chambers under controlled conditions. Maturation of fermented sausages is a complex process involving chemical, biochemical, physical-chemical and microbiological reactions between the ingredients of the charge, additives and components of smoke *(Vasilev, 2009; Karan et al., 2009; Rašeta et al., 2010).*

The production of fermented sausages gave especially important in the decline in pH during fermentation and maturation. This is achieved in two ways: the action of certain microorganisms and adding special additives *(Vuković et al., 1989).* Lactic acid bacteria regularly present in charge of raw sausages. In modern industrial production are increasingly using selected strains of bacteria lactic acid and some other microorganisms or starter cultures *(Luche and Hecelman, 1968; Bacus, 1982; Radetić, 1997).* Starter cultures perform fermentation of sugars using them as a source of energy while creating lactic and other acids that decrease the pH of the sausages (*Vesković - Moračanin et al., 2008).* Starter cultures are not yet found application in the production of fermented sausages in R. Macedonia.

The additives used in the manufacture of fermented sausages mostly used GDL. GDL causes a rapid drop in pH (towards the isoelectric point of aktomiozine) and fast release of water *(Koreti, 1971; Kuzelov et al., 2008).* The way he comes to quick release of water from the fast charge and binding components. The 5.2- 5.5 pH protein easily turn into a gel sheet that gives the required consistency sausages *(Vukovic 2006; Vuković et.all. 2011).*

In the last 20 years in the R. Macedonia has built more plants to produce meat. Some of them produce tea and pork sausage with added spices and GDL and various other products with trade names Tari S 77 receiving rapid, edelsausage primal, primal mediterano consisting of a GDL, ascorbic acid, sodium ascorbate, spices to flavor boosters, cookery salt etc. All these preparations contain in addition a means of reducing the pH and rapidly maturing of sausages and other durable assets in different ratio and are relatively expensive. The purpose of this study was to investigate the impact of functional mix (which is prepared in the laboratory of a local industry in the R. Macedonia to replace Tari S 77) and starter cultures on the sensory properties of tea sausage.

Material and Methods

As a material for making tea sausage serve frozen pork first category (thigh) and second category pork (shoulder) before cuttering whose temperature was -3.7ºC and hard fat -12.5ºC. Frozen meat and TMT are cuttering to the

granulationofparticles 3 mm. After the charge cuttering temperature was 2.5 to 3.2 ºC. Charge was prepared from 35% pork first class 35% pork second category and 30%TMT (dorsal bacon). The additives are used nitrite curing salt (2.4%), sodium ascorbate (0.05%) ground black pepper (0.020%), garlic powder (0.010%) mixture of spicy tea (Kolinska) (0.55%). As a starter culture used commercial preparation F - SC111 Bactoferm product company Chr Hansen Denmark. This product contains a mixed culture composed of *Lactobacilus sakei and Staphyococus Camosus* frozen in dry form. *Lactobacilus sakei* causes rapid souring and *Staphyococus Camosus* causes stable color and a mild aromatic flavor.

Charge is prepared in three variants: I 0.7% simple sugar sucrose (without Tarim S77 and functional mix) control sample, II Starter Culture 25g/100 kg. III 0.3% functional mix of durable sausages composed of GDL, Dextrose and Vitamin C. Functional mix is prepared in laboratory conditions in a meat industry in the R. Macedonia. Thus prepared charges are stuffed with vacuum pump in collagen wrapper diameter of 36 mm and pairs with a length of 30 cm. Upon filling all varieties of Tea sausage are displayed in the same mode of smoking and drying in air chambers for a period than 21 days.

During the drying and fermentation of sausages is measured temperature and pH 8 days early every day and 21st days. Aw value is measured 8 and 21 days. At the end of the manufacturing process is performed sensory analysis of the finished product. The value of pH was measured with pH potentiometric - EBRO HT 810 meter with a combined electrode probe thermometer and built for simultaneous measurement of pH and temperature. Aw value is measured by aw - meter Aqyalab (Wasington). Loss of weight (Callow) during ripening of sausages is determined by the difference in mass between the two measurements in the technological process and is expressed as a percentage relative to the initial mass.

Sensory analysis of finished products is performed according to the method of the ninth - grader scale scoring method developed by VNIIMP – Moscow (All-Russian Scientific Research Institute of Meat indystry) . Assessment of sensory properties was performed by7 experienced professionals. Evaluated following sensory attributes: appearance of sausage, looks at the intersection consistency, smell, taste, color fastness results. The test is processed by mathematical statistical method ANOVA MICROSOFT EXEL 2010-2013th.

Results and Discussion

The results of the examination of the dynamics of pH Tea flask are given in Table1.

Table 1. Dynamics of pH Tea sausage

Days of ripening	Variants		
	I $\overline{X}$ ± Sd	II $\overline{X}$ ± Sd	III $\overline{X}$ ± Sd
0	5.92 ± 0.07	5.88 ± 0.05	5.83 ± 0.04
1	5.88 ± 0.05	5.62 ± 0.08	5.58 ± 0.02
2	5.82 ± 0.05	5.58 ± 0.04	5.50 ± 0.07
3	5.78 ± 0.04	5.47 ± 0.07	5.43 ± 0.05
4	5.75 ± 0.02	5.37 ± 0.05	5.37 ± 0.04
5	5.72 ± 0.05	5.30 ± 0.04	5.30 ± 0.08
6	5.68 ± 0.07	5.27 ± 0.07	5.24 ± 0.05
7	5.65 ± 0.04	5.24 ± 0.05	5.20 ± 0.04
8	5.62 ± 0.08	5.19 ± 0.03	5.14 ± 0.07
21	5.59 ± 0.05	5.25 ± 0.02	5.10 ± 0.05

$\overline{X}$= mean, Sd = standard deviation.

The table shows that the initial values of pH in all varieties ranging from 5.83 to 5.92. By the 8th day of ripening and drying are determined lowest pH values in all three variants (5.62; 5.19; 5.14). In trials with starter culture and functional mix in the first day of registration fastest drop in pH (from 5.88 to 5.62 and from 5.83 to 5.58) compared with the control sample (from 5.92 to 5.88). The results of the measurement of pH obtained in the survey are consistent with results obtained (*Stamenković et al., 1990)* who found that Tea sausage produced with the addition of GDL pH declined faster and were eventually registered lower values (5.20) compared with Tea sausage which has only added sugars (5.68). The results of the examination of the activity of water in all three variants are given in table number 2.

Table 2. Average aw -values Tea sausage

Variants			
Days of ripening	I $\overline{X}$ ± Sd	II $\overline{X}$ ± Sd	III $\overline{X}$ ± Sd
8	0.920 ± 0.01	0.912 ± 0.02	0.910 ± 0.02
21	0.880 ± 0.03	0.838 ± 0.05	0.848 ± 0.05

$\overline{X}$= mean, Sd = standard deviation.

The table shows that on the 8th day of production of sausages aw ranged from 0.910 in the variant III to 0.920 in variant I. Somewhat larger decline was observed invariant II (to 0.074) versus variant III (to 0.062) and variant I (about

0.040). Aw differences are small and not statistically significant. Our results are consistent with the results of *(Ambrosidias et al., 2004; Kozacinski et al., 2008)* who found greater and more rapid decline in aw variants tea sausage made with GDL in terms of variants produced with starter culture sand plain sugar. The loss of weight (Callow) during drying and ripening of Tea sausage on 8 day of production is 28.5 to 29.85 (Table 3). There were no statistically significant differences in weight loss eighth day of production in all there groups Tea sausage.

Table 3. Loss of mass during drying and fermentation Tea sausage.

Variants			
Days of ripening	I $\overline{X}$ ± Sd	II $\overline{X}$ ± Sd	III $\overline{X}$ ± Sd
8	28.50 ± 0.25	29.85 ± 0.20	28.84 ± 0.18
21	38.60 ± 0.58	36.35 ± 0.15	37.04 ± 0.14

$\overline{X}$= mean, Sd = standard deviation;

The 21 days of production is determined loss of mass 36.35 to 38.60%. Statistical processing of the data showed that there were no statistically significant differences in loss of mass in all three variants tea sausage. The results we obtained are consistent with results obtained *(Šutić et al., 1990)*. They found loss of mass in the production of tea sausage produced in Tari S 77 of 36.16%. Based on there results of sensory testing Tea sausage can be seen that the lowest score of all tested sensory features a variant with added sugar and sensory bests cores in all sensory attributes studied variants produced with the addition of starter culture (Table 4).

Table 4. Sensory evaluation of Tea sausage

Variants			
Sensory properties	I $\overline{X}$ ± Sd	II $\overline{X}$ ± Sd	III $\overline{X}$ ± Sd
Appearance	7.52 ± 0.037	8.52 ± 0.31	7.59 ± 0.35
Appearance of intersection	7.06 ± 0.36	8.98 ± 0.037	8,62 ± 0.37
Consistency	6.29 ± 0.39	8.51 ± 0.28	7.85 ± 0.25
Color	6.15 ± 0.35	8.80 ± 0.33	8.20 ± 0.30
Scent	6.53 ± 040	8.90 ± 0.37	7.98 ± 0.35
Taste	6.75 ± 0.42	8.98 ± 0.29	8.20 ± 0.22
Overall acceptability	4.12* ± 0.48	8.52* ± 0.20	7.95* ± 0.28

$\overline{X}$= mean, Sd = standard deviation

*Statistical significant deferences p<0,01

The variant with added sugar was evident felled wrapper the surface and darker color on the surface and the intersection unlike variants with the addition of starter culture having good consistency, attractive color to the surface of the sausage section of hopes without cavities and appearance cracks in hopes. The overall acceptability between the (I) first and second (II) group (4.12 - 8.52) and between the first and the third group (4.12 - 7.95) are statistically significant differences ($p < 0.01$). There are no statistically significant differences between the other sensory attributes tested in all three variants studied tea sausages ($p > 0.05$).

The results in terms of sensory testing of all three variants tea sausage are consistent with the results obtained *(Radetić, 1997; Ambrozidias et al., 2004; Morettia et al., 2005; Sincic et al., 2006)* who studied sensory properties of tea sausage. They noted improved sensory properties in tea sausage produced with the addition of starter cultures.

Based on the results of sensory analysis and the results of other authors can conclude that the use of starter cultures in the production of tea sausage gives products with good sensory characteristics and functional mix is a good substitute for Tari S-77.

Conclusion

From executed tests and results can be derived the following conclusions:
-Initial values of pH in all varieties ranging from 5.83 to 5.92. By the 8th day of ripening and drying are determined lowest pH values in all three variants (5.62; 5.19; 5.14). In trials with starter culture and functional mix in the first day of registration fastest drop in pH (from 5.88 to 5.62 and from 5.83 to 5.58) compared with the control sample (from 5.92 to 5.88).

- Functional starter culture and mix showed no influence of the Tea loss of mass sausage
  Tea sausages made with the addition of starter culture had better sensory properties compared with tea sausage produced with the addition of functional mix and sucrose.
- Functional mix is a suitable replacement for Tari S-77. It can be prepared in any plant before production of durable sausages and cheap in the Tari S-77.

Uticaj funkcionalnog miksa i starter culture na senzorne osobine trajnih kobasica proizvedenih u industriskim uslovima

A.Kuzelov, D. Naseva, N. Taskov, D. Saneva, D. Spasova, D. Andronkov

Rezime

Cilj našeg istaživanja e uticaj funkcionalnog miksa (sastavljen od GDL, askorbinske kiseline i vitamin C) i starter culture (komercijalni preparat F – SC 111 Bactoferm proizvod firme Chr Hansen Danska). Ovaj preparat sadrži mesanu kulturu sastavlenu od *Lactobacilus sakei u Staphyococus Camosus* u smrznutoj suhoj formi. Izradjeni su tri varijante čajne kobasice i to: I – sa dodatkom obicnog šečera saharoze koja nam je posluzila kao kontrolna proba; II - sa dodatkom starter culture; III – sa dodatkom funkcionalnog miksa za trajne kobasice. Najbolje rezultate u odnosu na senzorne osobine imala je proba proizvedena sa dodatkom starter kultura a najslabije proba proizvedena sa dodatkom saharoze.

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ANIMAL HEALTH CONTROL OF PIGS ON COMERCIAL FARMS

J.Bojkovski[1], T. Vasiljević[2], A. Vasić[1], N. Zdravković[1], D. Stojanović[3], D. Rogožarski[4]

[1]University of Belgrade, Faculty of Veterinary Medicine, Belgrade, Serbia
[2]Delta Agrar, Serbia
[3]Scientific Veterinary Institute „Novi Sad“, Novi Sad, Serbia
[4]The veterinary specialistic institute Požarevac, Serbia

Abstract: The concept of modern industrial production of pigs on commercial farms is based, among other things, on the implementation of biosecurity measures, as well as solving problems of environmental protection, which greatly burden the production. It is well known that good health is a prerequisite of good pig reproduction or successful and profitable production of pig. The health status of the herd depends on many factors, such as the maintenance technology, nursing, nutrition, organization, level of staff training and the systematic implementation of health care. Today we are witnessing a large number of bacterial diseases, diseases of viral etiology and certain parasites that can seriously affect the production of pigs in intensive farming. These diseases require applying prophylactic and therapeutic measures, as well as increased surveillance of professional services to keep under control. Biosecurity, welfare, good manufacturing practices and risk analysis at critical control points are very important elements in intensive pig production and using of biosecurity measures is critical to protecting the health and success of the production.

Key words: swine, breeding diseases, biosecurity, ecology

Introduction

In intensive pig farming, there are more valid parameters that can be used to show the success or profitability of production, such as the number of live or weaned piglets, daily growth, duration of the fattening period, the number of unproductive day’s sows, etc. Today, the usual production of pigs on commercial farms would present number of weaned piglets and fattening pigs per sow delivered during the calendar year. The production parameter varies considerably between countries, with more or less developed swine industry. In order to improve

pig production on the farm is important to provide a good health of cows and pigs first days after farrowing (*Radojičić et al., 2002; Bojkovski et al ., 2005, 2011).*

In this review paper we present a summary of our years of research pertaining to solving reproductive health and biosecurity issues on industrial swine farms as well as an overview of environmental contaminants that were present on farms with some of the possible solutions. Flexible cooperation of farm holders with professional services with respect and implementation expertise, and applying a series of biotechnical measures and putting the emphasis on prevention of disease in order to promote good health of pigs, it is possible to improve the welfare of pigs and actual production (*Hristrov et al., 2006, 2008).*

The most common health and reproductive health problems on a commercial pig farm

In intensive pig production control of herd reproduction is the primary task. It is known that in comparison to other types of domestic animals, pigs are characterized by very high reproductive potential, considering that they sexually mature early, high ovulation rate, the period of gestation and lactation which are relatively short, fast establishment of gestation after weaning the previous litter. From the economic point of view, proper, regular reproductive activity of pigs is of a great importance. If one herd reproductive efficiency satisfactory, is usually estimated on the basis of: age at which females farrow for the first time, the length of their reproductive exploitation, the duration of the interval between individual farrowing rate and litter size in weaning. Reproductive activity in pigs is influenced by many factors, among which are very important: hereditary factors, endogenous factors (hormones, immunoglobulins, enzymes), the influence of the environment, the presence of pathogens, as well as management and production technology (*Uzelac, Vasiljević, 2011.*). These additional factors also affect the reproductive efficiency: the gait, nutrition, season, farm location, microclimate, implementing biosecurity measures, herd size, herd health status (presence of breeding, parasitic and infectious diseases), body condition, the method of application of artificial insemination (*Lončarević et al., 1997, Petrujkić et al. 2011*). The problem of infertility is a common problem on commercial farms. The causes of infertility are various and numerous. Current problem of most of our farm is the emergence of seasonal infertility that is present during the summer months and is a serious impediment to producers who want to maximize their reproductive efficiency of the herd (*Petrujkić et al., 2009, 2010, 2011)*. In this sense, in intensive pig production much attention is now paid on devoted optimum of microclimate conditions in facilities for housing, using computerized systems for ventilation, cooling, lighting, water, feeding, manure with programmable desired parameters at

specific time intervals, thus creating animals the most favorable conditions to maximally express their genetic potential, achieve high productivity and greatly reduce the stress. Adequate health care for farm animals, a high level of hygiene of animals, equipment and people as well as the precise application of all procedures in the technology of artificial insemination are the primary requirements for high reproductive efficiency of breeding animals *(Stančić et al. 2012*).

Conventional assessment of the quality of semen, as a segment of technology of artificial insemination, is practiced largely on our commercial farms. Classical assessment of the seed in commercial terms can be used to recognize ejaculates with low potential for fertilization, but does not achieve high efficacy in predicting fertility parameters in the field (*Tsakmakidis, 2011*). Therefore, in order to combat infertility and control of reproductive efficiency of pigs today are implemented successfully, in cooperation with the Institute, laboratory methods, such as motion estimation using computer analysis (CASA), the automatic analysis of sperm morphology (ASMA), determination of the integrity of chromatin via flow cytometry, HOS test, etc. In this way, fertility of boars can be continuously monitored and it enables promptly reaction to the immediate production. Technology preparations of heterosperm insemination doses involving sperm of two or more races of terminal boars also found use in artificial insemination on our commercial farms to produce more piglets per sow (*Vasiljević, 2012*)

The use of deep frozen seed is also used in the world on industrial swine farms. The advantage of deep frozen seed is that it keeps the genetic material of a longer time period and significantly reduces the risk of introduction of the disease in the herd (*Stankovic et al.,2007)*. However, the deep freeze did not enter into wide practice because technologies of deep freeze have not been resolved in a satisfactory extent and a low percentage of pregnancy and litter size is lower (*Vidović et al., 2011*.). The phenomenon of stress is also one of the serious problems in commercial farms. Farms that are still developing their management have a greater problem with stress than farms that have organized complete production. The requirements of modern pig production today are reduced stress to the minimum and provide maximum comfort of animals (welfare). In this regard it is important to know and consider mechanisms for adaptation syndrome and stress reactions and to provide to the animals more adequate living conditions in order to expect productivity on desired level. High levels of corticosteroids in the blood of animals exposed to stress are acting to reduce their resistance and thus allowing them to become highly susceptible to various infections. Therefore it is very important to promote animal welfare in farm conditions through the development and improvement of man's consciousness in the direction of respect, caring and responsibility towards animals, as well as the application of technical and

technological solutions in the production of animals that will provide maximum comfort and convenience.

Technology of feeding of farm animals also plays a significant role in the prevention of stress and is also a very important factor maintaining high health and reproductive status. Too much fattened sows as they carry a large number of offspring, and in addition consume a large amount of food in facilities where the increased humidity and temperature exist, are more susceptible to stress and show signs of respiratory distress. It's one of the reasons for the introduction of recommendations related to diet by production stages and categories of animals. Precisly is defined, for example the feeding curve of breeding sows at each stage of production in order to facilitate the early entry into estrus after weaning of piglets, a large number of super ovulated and implanted embryos, a greater number of live, vital piglets, the greater the amount of the produced milk during lactation and that at the same time is possible to preserve the fitness and health of cows in order to live longer and have longer productive life and to lower the use of medications. Thanks to this approach, today is nothing new that we have commercial farms with 35 and more weaned piglets per sow per year.

Production of pigs on commercial farms is largely burdened by diseases of piglets. Pathology of piglets is a very dynamic discipline within the entire herd epizootiology in which the large agglomerations of animals in a confined space can easily come up with horizontal and vertical transmission of infection and certain microorganisms due to the forced cultivation and possession of lead to the production and technological disease. Great importance is attached to the variations of pathogens in pigs, not only in showing resistance to drugs, but the occurrence of genetic recombination, which affect the clinical picture and course of the disease, all of which makes it difficult to diagnose and apply therapy and prophylaxis *(Blackburn, 1995, Bojkovski et al., 1997, 2005).* In our pig farms are present: Neonatal colibacillosis, endemic disease, necrotic enteritis, circoviral infections, colitis caused by spirohaetes, enterohaemorrhagic syndrome, dysentery and respiratory disease complex. In recent years, in the world and on our pig farms there was a mass occurrence of respiratory disease complex (PRDC), which is becoming a serious health problem in all technological stages of production. The complex is a respiratory disease of pigs characterized by simultaneous infection of lung tissue with more respiratory pathogens and is a common term for pneumonia in pigs with multifactorial etiology. Isolated pathogens vary between and within production herds (*Honnold, 1999; Ivetić et al., 2005; Golinar et al. 2006).* Control of respiratory disease complex is difficult and complicated. The significance of respiratory diseases complex is based on the interaction of respiratory pathogens. Knowledge of the interaction of respiratory pathogens should be considered in order to implement effective control measures. Respiratory disease of pigs occurs if

the causative agents live in the same habitat or if due to an unknown cause for us, the immune response mechanisms of respiratory system weakens (*Ivetić et al. 2005).* Unlike classical spreading control of infectious diseases of pigs that persist in our country, and whose fighting a legal obligation, detection and suppression technopathy is more an economic need of the producers themselves.

Cytogenetic methods

In modern pig production genetics aims to improve the productive capacity of existing breeds that are used on farms of industrial type, creating new forms of quality races with higher genetic potential and the growing of pure breed or crosses for commercial purposes. One part of our research has been focused on the research of changes in karyotype pigs in intensive education. We found that changes in the karyotype may occur under the influence of chemicals, which can be found in food, water, or in the general environment in which test animals live (*Bojkovski, 2010*). Our recommendation is that industrial-type farms and centers for reproduction and artificial insemination apply the results of cytogenetic methods, which allow detection of carriers of hereditary anomaly. With their involvement in farm biosecurity plans there can be positive influence on the health of the herd and improvement of production results.

Ecological problems on commercial pig farms

In the long period on the big commercial pig farms attention has been paid on the presence of chemical environmental pollutants (heavy metals) and their impact on the health of the animals. Special danger to living systems are heavy metals that react with organic molecules changing their structure and function. Heavy metals penetrate in the organism through the respiratory, digestive tract and skin. The results of years of research have pointed to the risk of contamination of animal feed with heavy metals and their deposition in the body of animals, with consequences on the health and reproductive capacity of animals. The toxicity of heavy metals generally lead to the formation of free radicals, inhibiting the activity of antioxidant enzymes and oxidation of glutathione, and the creation of malonyl-dialdehyde (MDA) as a marker of oxidative stress. Their toxicity comes from the tendency to create links with covalent sulphydrile groups, biomacromolecules or extruded certain cofactors, which inhibit the activity of certain enzymes *(Bojkovski et al., 2008a, b, 2010a).* Our recommendation for farms of industrial type on which is necessary to act to reduce the risk of the effects of heavy metals, is working on the introduction of

multiple monitoring the quality of raw materials and finished products, as well as the application of adequate protectors of the toxic effects of these agents *(Bojkovski et al, 2010b, c)*.

Biosecurity on commercial pig farms

Biosecurity plans are key to disease prevention, prevention of unwanted situations and business improvement *(Uhlehoop, 2007)*. The global objective of the contemporary swine developed countries is to prevent the entry of disease into the herd that is disabling to the fullest extent that the pigs come in contact with infectious agents in the environment as well as prevent or minimize the transfer of pathogenic germs within the herd, between certain categories of animals. Therefore, special attention is paid to the technical solutions that make it possible to protect the herd of pigs and keep it isolated from harmful external influences such as the construction of quarantine ward for newly acquired animals, the formation of a separate department for the delivery of animals as well as the input for the personnel department with the prescribed hygiene measures and protocol behavior. All measures that aim to protect the herd from infection are called biosecurity measures and include measures of external and internal biosecurity defined by biosecurity protocol. Meaning that external biosecurity disables the transmission of infectious agents in the environment, and other herds in the region. It comprises multisite system of accommodation, control entry into the farm (manpower, animal feed, equipment, materials, and seeds for artificial insemination.), control of the vehicle around the farm, control of rodents, insects and birds, protocol for entry of employees, animal control supplies, procedure with dead animals, and quarantine of newly purchased animals. Internal biosecurity includes procedures for entry and conduct of employees within the farm (showering, farm clothes and shoes, moving through the farm people and animals, the use of tools and accessories for work ..), the principle of "all in - all out" protocol cleaning, washing and disinfection and infection control through a program of preventive and curative health care of animals *(Uzelac, Vasiljevic, 2011.)*. Evaluation of biosecurity on the basis of indicators should be routine in the evaluation of biosecurity on farms, pointing the way to act and, possibly, their improvement *(Lončarević et al., 1997, Stankovic et al., 2008)*. For example working on reviewing failures in obtaining biosecurity Stankovic and Hristov (2009) argue that the level of biosecurity on a study of pig farms was estimated to 3.96 (very good). This result indicates the current state of biosecurity farm, but must always bear in mind the mutual interaction and totality of action parameters of biosecurity *(Stankovic and Hristov, 2009)*. Breeders have the primary responsibility to protect their own herds in terms of introduction of the disease

control movement, proper procedure and accommodating groups of animals and sanitation. Employees on the farm as well as visitors must be aware of their role in maintaining a safe health status of the farm *(Stankovic and Hristov, 2009)*

Conclusion

The goal of intensive production of pigs on commercial farms is to produce a large number of weaned piglets and fattening pigs per sow per year. To achieve this goal it is necessary to establish a high reproductive efficiency of breeding animals. This can be achieved by an appropriate health care, advanced technology and good organization of production with the use of appropriate procedures in the technology of artificial insemination.
Efforts are paid in production today in commercial farms that the phenomenon of stress is reduced to the lowest possible level.

High health status and health control programs using health care through preventive and curative measures and a protocol of external and internal biosafety is imperative in manufacturing.

Our recommendation is that industrial type farms and centers of reproduction and artificial insemination approaches to the introduction of cytogenetic methods that allow the detection of carriers of hereditary anomalies. In order to reduce the risk from the effects of chemical pollutants of the environment, it is necessary to work on the introduction of multiple quality monitoring of raw materials and finished products as well as the application of adequate protector of the toxic effects of these agents.

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Kontrola zdravlja stada svinja na komercijalnim farmama

J.Bojkovski, T. Vasiljević, A. Vasić, N. Zdravković, D. Stojanović, D. Rogožarski

Rezime

Koncept savremene industrijske proizvodnje svinja na komercijalnim farmama zasnovan je, između ostalog i na sprovođenju biosigurnosnih mera, kao i

na rešavanju problema ekološke zaštite koji u velikoj meri opterećuju proizvodnju. Poznato je da dobro zdravlje svinja predstavlja preduslov dobre reprodukcije, odnosno uspešne i profitabilne proizvodnje u svinjarstvu. Zdravstveni status stada zavisi od velikog broja činilaca , kao što su su tehnologija držanja, nege, ishrane, organizacija, stepen obučenosti kadrova kao i sistematsko sprovođenje mera zdravstvene zaštite . Danas smo svedoci da veliki broj oboljenja bakterijske, virusne etiologije kao i pojedine parazitoze mogu ozbiljno ugroziti proizvodnju svinja u intenzivnom uzgoju. Ove bolesti moguće je primenom profilaktičkih i terapeutskih mera, kao i pojačanim nadzorom stručnih službi držati pod kontrolom. Biosigurnost, dobrobit, dobra proizvođačka praksa kao i analiza rizika na kritičnim kontrolnim tačkama su veoma značajni elementi u intenzivnoj proizvodnji svinja a planska primena biosigurnosnih mera presudna je u zaštiti zdravlja i uspehu proizvodnje.

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WHICH AMOUNT OF TOTAL ENERGY REQUIREMENT OF SUCKLING PIGLETS CAN BE COVERED BY MILK OF SOWS?

J. Kecman, M. Waehner

Anhalt University of Applied Sciences, Strenzfelder Allee 28, 06406 Bernburg, Germany
Corresponding author: jelena.kecman@gmail.com
Original scientific paper

Abstract: In the last years the number of piglets born per litter increased to more than 14 piglets. Therefore the sows need to be able to produce more milk. In a study milk yield of 48 sows of Topigs origin was estimated once a week during a 4-week lactation using the method "weigh-suckle-weigh". The milk intake of piglets and consequently milk yield of sows were corrected for weight loss of piglets by urine and feces. Furthermore, it was assessed which amount of total energy requirement of the piglets could be covered by the milk of sows. For this purpose the energy content of the milk and the total daily energy requirement of the piglets were estimated. The daily milk yield of sows increased from 5.4±1.3kg milk in the 1st week of lactation by 8.9±2.4kg in the 2nd week to 10.5±2.4kg in the 3rd week of lactation. In the 4th week of lactation the daily milk yield decreased to 8.8±2.0kg milk. In the 1st week of lactation the milk of sows contains energy at an amount of 30.3±7.9 MJ ME/day. So the total energy requirement of the piglets of 28.4±7.8 MJ ME/day and litter are covered by milk completely. The conditions for that are a daily maintenance requirement of piglets of 0.440 MJ ME per kg $BW^{0,75}$ and a litter size of less than 13 piglets. The negative energy balance of the piglets needs to be compensated from the 2nd until the end of 4th week of lactation by creep feeding.

Key words: milk yield of sows, energy requirement of piglets

Introduction

In recent years the milk yield of sows has increased. Nowadays sows are able to produce more than 10 kg milk per day depending on genetics, week of lactation, litter number, litter size, feeding, body conditions e. g. *(King and Eason, 1998; Ramanau, 2004; Kecman et al., 2013)*. With the increase of the number of piglets per litter, the question arises: which amount of total energy requirements of suckling piglets can be covered by milk of sows? In literature only a few data can

be found for this topic. This refers especially to the total energy requirements of piglets in the first two weeks of lactation period.

The aim of a study was to estimate the milk yield of sows during a 4-week lactation period and to assess which amount of total energy requirement of piglets could be covered by the milk of sows.

Material and Methods

The study started in October 2011 in State Institute for Agriculture, Forestry and Horticulture Saxony-Anhalt and was finished in December 2012. The milk yield of 48 sows of Topigs origin was estimated once a week during 4-week lactation period (on days 4., 11., 18., and 25. of lactation) using the method "weigh-suckle-weigh". Daily milk yield was calculated by multiplying the average milk intake (MI) of piglets per litter during one suckling act (SA) with the number of suckling acts per day. Die number of suckling acts was 24 times per day in the 1st, 2nd and 3rd week of lactation *(Kecman et al., 2012)* and 20 times per day in the 4th week of lactation *(Smith, 1952; Barber et al., 1955; Hartman et al., 1962; Noblet and Etienne, 1989)*. To obtain the precise values, milk intake of piglets and consequently milk yield of sows was corrected for weight loss of piglet by urine and feces. This correction was performed using the frequencies of urination and defecation of piglets during the suckling period and the amount of urine and feces in each week of lactation. Therefore, the amount of urine and feces of piglets was collected in each week of lactation. Concerning the urine, the milk intake of piglets was corrected with a value of 15.9 g in the 1st week to 47.7 g in the 4th week of lactation respectively with a value of 1.3 g to 5.0 g concerning the feces to the same times (Table 1).

Table 1. Urinary and fecel losses of piglets during lactation period

Parameter, n=273	Week of lactation			
	1st	2nd	3rd	4th
Amount of urine, g	15.9 ± 8.0	30.3 ± 14.5	38.6 ± 15.1	47.7 ± 26.9
Amount of feces, g	1.3 ± 0.4	2.5 ± 1.2	3.5 ± 1.7	5.0 ± 1.9

The individual milk samples were taken from sows in the 1st, 2nd and 3rd week of lactation period for analyzing the fat, protein and lactose content. The analysis was carried out using Milko Scan™ FT + and was performed in the central laboratory of the State Control Association for Performance and Quality Inspection of Saxony-Anhalt in Halle. The energy content of milk of sows was calculated with the formula by *Klaver et al.* (1981):

MJ ME/kg milk = 0,256 x % protein + 0,386 x % fat + 0,149 x % lactose.

The piglets were weighed at birth and at the end of each weeks of lactation period. The maintenance requirement of piglets was calculated using a daily energy requirement of 0.440 MJ (*GfE, 2006*) metabolic energy (ME) per kg of metabolic body weight ($BW^{0,75}$) respectively 0.645 and 0.725 MJ ME per kg $BW^{0,75}$ *(Jeroch et al., 1999)*. The energy requirements of piglets for performance was calculated on average 1.1 MJ ME per 100 g weight gain *(LfL, 2008).* Feeding of the piglets with creep feed was done from day 12 of lactation until weaning. The energy content of creep feed (prestarter) was 15.6 MJ ME / kg.

Results and Discussion

The average milk intake (MI) of piglets per litter during one suckling act (SA) ranged from 224.7 ± 55.0 to 439.2 ± 100.7 g milk (Figure 1).

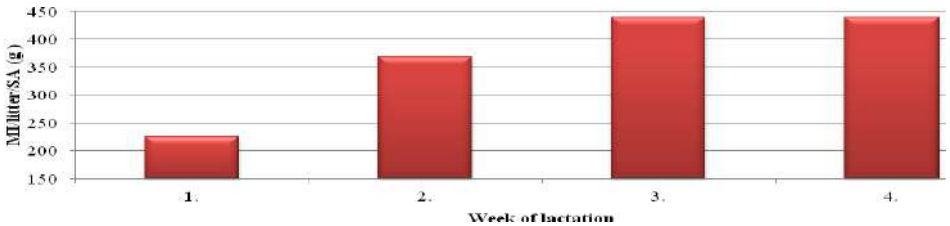

Figure 1. Average milk intake of piglets per litter during one suckling act depending on week of lactation (g)

The average daily milk yield of sows increased from 5.4 ± 1.3 kg in the 1st week of lactation by 8.9 ± 2.4 kg in the 2nd week to 10.5 ± 2.4 kg in the 3th week of lactation. In the 4th week of lactation daily milk yield decreased to 8.8 ± 2.0 kg. The milk had the highest energy content (5.6 ± 0.6 MJ ME/kg) in the 1st week of lactation. This resulted primarily from a higher fat and also protein content of the milk in this week. The energy content of milk per kg decreased throughout the lactation, so it was 4.9 ± 0.6 MJ ME/kg in the 3rd week (Table 2).

Table 2. Composition and energy content of milk of sows during lactation period

Parameter	Week of lactation		
	1st	2nd	3rd/4th*
Fat content, %	$8.9^{a} \pm 1.6$	$8.4^{a} \pm 1.2$	$7.3^{b} \pm 1.3$
Protein content, %	$5.5^{a} \pm 0.6$	$5.0^{b} \pm 1.0$	$4.8^{b} \pm 0.5$
Lactose content, %	$5.3^{a} \pm 0.4$	5.5 ± 0.5	$5.6^{b} \pm 0.6$
Energy content, MJ ME/kg milk	$5.6^{a} \pm 0.6$	$5.4^{bc} \pm 0.5$	$4.9^{bd} \pm 0.6$

a,b,c,d- mean values in rows followed by the same letters are significantly different at $p<0,05$
* The results for fat, protein and lactose content in the 3rd week were used also in the 4th week of lactation.

Based on the daily milk yield and the energy content of milk/kg the daily energy content was calculated of milk of sows (MJ ME/day). It was 30.3 ± 7.9 MJ ME/day in the 1st week of lactation respectively 47.2 ± 12.3 in the der 2nd week, 51.6 ± 13.9 in the 3rd week and 42.7 ± 11.0 MJ ME/day in the 4th week of lactation.

The average daily weight gain of piglets was 267.21 ± 80.68 g. In the 2nd week of lactation the piglets were achieved the highest daily weight gain of 305.9 ± 59.7 g (Figure 2).

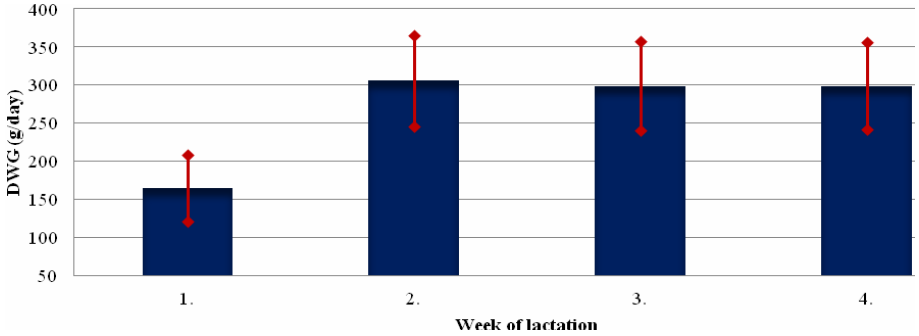

Figure 2. Average daily weight gain of piglets depending on week of lactation (g/day)

Based on this daily weight gain (Figure 2) the weight gain of a whole litter was estimated. In the 1st week of lactation a litter weight gain of 11.54 ± 3.02 kg was achieved. In the second week this was 21.49 ± 4.24 kg, in the 3rd week 20.93 ± 4.10 kg and in the 4th week 20.89 ± 4.06 kg (Table 3).

Table 3. Weight gain of litter, weights of piglets and litter size during lactation period

Parameter	Week of lactation			
	1st	2nd	3rd	4th
Weight gain, kg/litter	11.54 ± 3.02	21.49 ± 4.24	20.93 ± 4.10	20.89 ± 4,06
Weight, kg/piglet*	1.99 ± 0.22	3.49 ± 0.42	5.45 ± 0.66	7.41 ± 0.84
Litter size	11.58 ± 1.41	11.35 ± 1.34	11.17 ± 1.37	11.08 ± 1.38

* Piglet weights in the middle of each weeks of lactation - used to calculate maintenance energy requirement of piglets.

A piglet with an average weight of 1.99 ± 0.22 kg in the 1st week of lactation (Table 2) needed 0.74 ± 0.06 MJ ME/day to cover the maintenance requirements if daily requirements of 0.440 MJ ME/kg $BW^{0,75}$ were assumed (Table 4). By daily maintenance requirement of 0.625 MJ ME the same piglet would need 1.08 ± 0.09 MJ ME/day respectively 1.21 ± 0.10 MJ ME/day by daily maintenance requirement of 0.725 MJ ME/kg $BW^{0,75}$. The daily maintenance requirement of piglets increased in each week of lactation with increasing weights of piglets (Table 4).

Table 4. Energy requirements of piglets for maintenance and weight gain during lactation period (MJ ME/day & piglet)

Week of lactation	Energy requirements for maintenance (per kg $BW^{0,75}$)			Energy requirements for weight gain MJ ME
	0.440 MJ ME	0.645 MJ ME	0.725 MJ ME	
1st	0.74 ± 0.06	1.08 ± 0.09	1.21 ± 0.10	1.69 ± 0.44
2nd	1.12 ± 0.10	1.64 ± 0.15	1.85 ± 0.17	3.71 ± 0.72
3rd	1.57 ± 0.14	2.30 ± 0.21	2.58 ± 0.23	3.44 ± 0.67
4th	1.97 ± 0.17	2.89 ± 0.24	3.25 ± 0.28	3.44 ± 0.67

The energy requirements of piglets for performance were calculated on average 1.1 MJ ME per 100 g weight gain. The average daily weight gain of piglets in the 1st week of lactation period was 164.9 ± 43.1 g (Figure 2). To achieve this result a piglet needs 1.69 ± 0.44 MJ ME/ day. In the 2nd week of lactation the average daily weight gain is 305.9 ± 59.7 g and the piglet requires 3.71 ± 0.72 MJ ME/day. In the 3rd and 4th week of lactation the daily weight gain of piglets was at the same level (3th week: 299.0 ± 58.6; 4th week: 299.0 ± 57.4 g / day). The energy requirement for this daily weight gain was 3.44 ± 0.67 MJ ME/day/piglet (Table 4).

Based on the energy requirements for maintenance and performance total energy requirement of piglets was calculated (Table 5).

Table 5. Energy content of milk of sows and total energy requirements of piglets during lactation period (MJ ME/day & litter)

Week of lactation	Milk MJ ME/day	Total energy requirements of piglets/litter		
		0.440 MJ ME	0.645 MJ ME	0.725 MJ ME
1st	30.3 ± 7.9	28.4 ± 7.8	32.3 ± 8.2	33.9 ± 8.4
2nd	47.2 ± 12.3	55.3 ± 12.7	61.2 ± 13.5	63.5 ± 13.8
3rd	51.6 ± 13.9	56.2 ± 12.4	64.3 ± 13.4	67.5 ± 13.7
4th	42.7 ± 11.0	60.0 ± 11.5	70.2 ± 12.5	74.2 ± 12.9

By higher energy requirements for maintenance of 0.645 or 0.725 MJ ME/kg $BW^{0,75}$ and day (vs. 0.440 MJ ME) the total energy requirement of piglets increased. Generally, it increased with a growth of piglets in each week of lactation.

In the 1st week of lactation the milk of sows contains energy at an amount of 30.3±7.9 MJ ME/day. So the total energy requirements of the piglets of 28.4±7.8 MJ ME/day and litter are covered completely by milk. The conditions for that are a daily maintenance requirement of piglets of 0.440 MJ ME per kg $BW^{0,75}$ and a litter size of less than 13 piglets.

The difference between the energy content of milk of sows and the total energy requirement of piglets is represented by the energy balance of the piglets. The table 6 shows the energy balance of piglets during lactation period.

Table 6. Energy balance of piglets during lactation period (MJ ME/day & litter)

Week of lactation	Energy balance of piglets		
	0.440 MJ ME	0.645 MJ ME	0.725 MJ ME
1st	positive	negative	negative
2nd	- 8.14	- 14.06	- 16.38
3rd	- 4.61	- 12.75	- 15.92
4th	- 17.30	- 27.46	- 31.43

Only in the 1st week of lactation and only by daily maintenance requirement of 0.440 MJ ME the energy balance of piglets was positive. The negative energy balance of the piglets needs to be compensated from the 2nd until the end of 4th week of lactation by creep feeding. The amount of creep feed varied from 46 g to even 182 g/day and piglet (Table 7).

Table 7. Calculated amounts of creep feed during lactation period (g/day & piglet)

Week of lactation	Amounts of creep feed (g/day & piglet)		
	0.440 MJ ME	0.645 MJ ME	0.725 MJ ME
1st	-	-	-
2nd	46.0	82.5	92.5
3rd	26.5	73.2	91.4
4th	100.1	158.9	181.8

Conclusion

The daily milk yield of sows increased from 5.4±1.3kg milk in the 1st week of lactation by 8.9±2.4kg in the 2nd week to 10.5±2.4kg in the 3rd week of lactation. In the 4th week of lactation the daily milk yield decreased to 8.8±2.0kg milk. In the 1st week of lactation the milk of sows contains energy at an amount of 30.3±7.9 MJ ME/day. So the total energy requirements of the piglets of 28.4±7.8 MJ ME/day and litter are covered by milk completely. The conditions for that are a daily maintenance requirement of piglets of 0.440 MJ ME per kg $BW^{0,75}$ and a litter size of less than 13 piglets. The negative energy balance of the piglets needs to be compensated from the 2nd until the end of 4th week of lactation by creep feeding.

Koji iznos ukupnih energetskih potreba prasadi na sisi može da zadovolji mleko krmača?

J. Kecman, M. Waehner

Rezime

U poslednjih nekoliko godina broj rođene prasadi po leglu porastao je na više od 14 prasadi. Zato krmače moraju biti u stanju da proizvode više mleka. U ovom ispitivanju, prinos mleka od 48 krmača Topigs porekla utvrđivan je jednom nedeljno tokom 4 nedelje laktacije, koristeći metod "merenje-sisanje-merenje". Unos mleka prasadi, a samim tim i prinos mleka kod krmača su korigovani za gubitak mase prasadi od urina i fecesa. Pored toga, ocenjeno je koji iznos ukupnih energetskih potreba prasadi na sisi može da zadovolji mleko krmača. U tom cilju energetski sadržaj mleka i ukupne dnevne energetske potrebe prasadi su procenjeneni. Dnevna mlečnost krmača porasla je od 5,4 ± 1.3kg mleka u 1. nedelji laktacije na 8,9 ± 2.4kg u 2. nedelji i 10,5 ± 2.4kg u 3. nedelji laktacije. U 4. nedelji laktacije dnevna mlečnost smanjena je na 8.8 ± 2.0kg mleka. U 1. nedelji laktacije mleko krmača sadrži energiju u iznosu od 30,3 ± 7,9 MJ ME/dan. Dakle, ukupne

energetske potrebe prasadi od 28,4 ± 7,8 MJ ME/po danu i leglu mleko pokriva u potpunosti. Uslovi za to su dnevn potrebe prasadi za održavanje od 0,440 MJ ME po kg $BW^{0,75}$ i veličine legla manje od 13 prasadi. Negativan energetski bilans prasadi treba nadoknaditi od 2. do kraja 4. nedelje laktacije prihranjivanjem.

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CLUSTER ANALYSIS – A STATISTICAL TOOL TO ANALYSE THE COMPLEX RELATIONSHIP WITHIN DIFFERENT PARAMETERS IN PIGS

H. Schlegel, K. Fischer, M. Waehner

Anhalt University of Applied Sciences, Strenzfelder Allee 28, 06406 Bernburg, Germany
Corresponding author: hannes.schlegel@gmx.de
Original scientific paper

Abstract: In modern agriculture many data according to fertility of sows or fattening performance of pigs are analyzed. Comparatively new is the use of a cluster analysis to examine a high number of parameters at the same time. There are no conditions on scale or distribution of data. It is also possible to apply a cluster analysis even if you do not know anything about the relationship between the examined parameters. In order to demonstrate the application and the scope of a cluster analysis there are two examples of pig breeding. In the first example three parameters have been used for an optimal clustering. In result three and four clusters are interpreted in their meaning. A different number of cluster leads to a variety of different possibilities of interpretation. In this example the numbers of piglets born in total, born alive and the mean birth weight have been analyzed. If you use four instead of three clusters it is easier to interpret them and to make the right decision for breeding. The second experiment demonstrates the complexity of a cluster analysis. If you use a high number of parameters you need to be very careful to rate the first results and to conclude the right following steps for a correct clustering. In this case five parameters have been used in different ways of algorithms of fusion. In general cluster analysis is a useful tool to interpret your data, but you need to be very careful in handling this method.

Key words: hierarchic cluster analysis, pig breeding

Introduction

Sows of modern breed do have a high genetic potential. Important impact factors are the performance per litter, in this case the number of born and weaned piglets and the longevity of the sows. A high longevity and high numbers of weaned piglets per lifetime are the basis for en economic pig production. With the help of statistical methods different correlations between single and more parameters can be detected in complex.

It happens continuously to analyze single or a defined small number of parameters at once. The usage of a cluster analysis is relatively new. It is applied for the examination of a high number of impact factors, with know or unknown correlations between (*Fischer et al., 2010, Fischer et al., 2011*).

Materials and Methods

To demonstrate the practical use and the scope of a cluster analysis two examples have been chosen. Therefore data of sows from one farm but from two places are used.

The environmental conditions were equal and comparable. In the first example 406 sows from the 1st to the 11th litter have been analyzed according to the parameter average weight at birth of the piglets, number of total born piglets (TBP) and number of life born piglets (LBP). In the second more complex example 1.802 sows from the 1st to the 12th litter are examined according to the parameter of TBP, LBP and the used boar, the technician for insemination and the number of litter.

In general a cluster analysis is used as a method to detect structures within and between parameters and to group those parameters. This means that there are no known and no implemented correlations. Cluster analysis is divided into the analysis of centers of a cluster and into hierarchic methods. The analysis of centers of a cluster starts with a defined number of cluster and a random grouping. Then objects between the groups are changed until one finds an optimal solution. The hierarchic method uses a gradual process. After finishing of this cluster analysis sows of one cluster should have nearly the same characteristics in an optimal solution. Despite of that sows of different cluster should be very different in their parameters. The more they are different the easier it is to assign special attributes and to interpret the results *(Jansen et al., 2009*). Beside the possibility to calculate with a high number of parameters there is one big advantage that there are no needs to scale to parameters. The hierarchic method contains many different possibilities to adopt the results to the prepared data. It is very labor-intensive, but necessary to detect the different and hidden structures within the data. The process of the hierarchic method works in four phases.

In the **first step** the similarity or dissimilarity of the objects gets determined by the calculation of a matrix. Within this matrix all objects are compared to each other. Similarity between objects gets assigned by different scales of similarity for nominal values. One example for a nominal parameter is health status of an animal (yes or no). Dissimilarity between different objects gets assigned by different scales for distance for metric variables. Birth weight of piglets is an example for a metric variable. The result of clustering can be influenced decisive by the correct or incorrect choice of the scales for distance or

similarity. Therefore many possibilities should be tested (Eckey et al., 2002). Examples for scales of similarity are the Tanimoto coefficient or the simple matching coefficient. Examples for scales of distance are euclidic distance and the squared euclidic distance. The matrix of similarity and distance are the basis for the second step.

In the **second step** the algorithm of fusion for objects and clusters needs to be chosen. There are seven different algorithms within the program SPSS 19.0. Those algorithms work in different ways and on different starting points. But on this way they put objects or later on cluster together to groups. The result can be changed very much. If you use the average linkage algorithm all distances between the objects of two clusters are used for estimation. Means of all distances are calculated. The two clusters of objects with the lowest mean value are put together.

In the **third step** the optimal number of cluster gets determined by the user. This can be caused by the question which is given. In this case an optimal number of clusters don't need to be achieved. Another reason for a special number of clusters can be the interpretation of a dendrogram of a diagram of icicles. Both ways are practicable with a small number of objects. If it gets more, it will become more difficult.

In the **fourth step** the special features of the different clusters get assigned. Therefore you need additional methods, e.g. cross tables, means and standard deviation (*Backhaus et al., 2008*).

Results and Discussion

In the **first example** 406 sows get grouped. The parameters mean birth weight of piglets, number of total born piglets (TBP) and number of live born piglets (LBP) are considered. In addition to that the number of litters was determined to interpret the results of the cluster. As result there are two different variants as the best choice with three or four cluster. In the **first variant** there are 91 sows in cluster one, 194 sows in cluster two and 121 sows in cluster three. Table 1 shows you the values of the mean weight of the piglets, the number of total born piglets, the number of live born piglets and the number of litter according to the cluster.

Table 1. Values of mean weight of piglets, number of total born piglets, number of live born piglets and the number of litter according to cluster

parameter	cluster 1 (n = 91)	cluster 2 (n = 194)	cluster 3 (n = 121)
TBP (MW±SD)	15.6 ± 1.8[a]	13.2 ± 1.5[b]	9.5 ± 1.6[c]
LBP (MW±SD)	15.0 ± 1.6[a]	12.6 ± 1.3[b]	9.1 ± 1.6[c]
average birth weight in kg	1.3 ± 0.15[a]	1.5 ± 0.2[b]	1.6 ± 0.2[c]
number of litter (MV±SD)	3.8 ± 2.3	3,6 ± 2.5	3.2 ± 2.1

a:b:c:d p<0,05

The 91 sows of the 1st cluster do have a high number of TBP and LBP. The average birth weight of the piglets is lower than in the other groups. In the 2nd cluster the birth weight is higher and the number of piglets TBP and LBP are lower. In the 3dr cluster the sows achieve the best result for the average birth weight of the piglets but they reach the lowest results for TBP and LBP. This contrast between the number of piglets and the mean birth weight of piglets is demonstrated in figure 1.

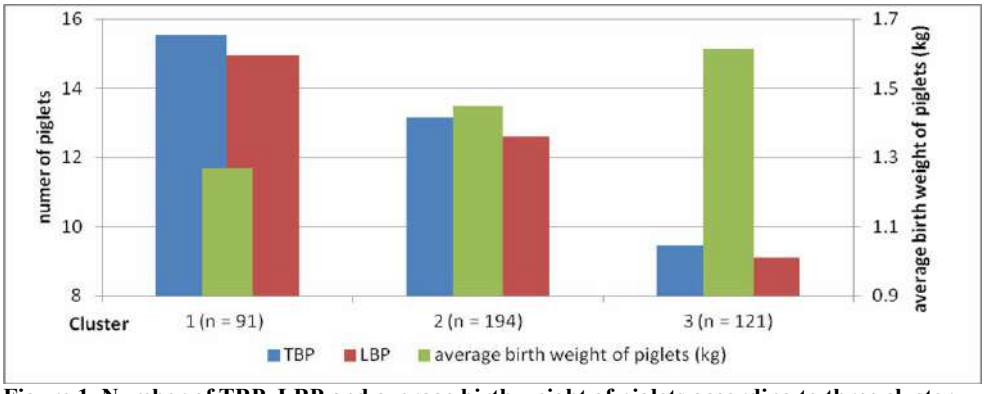

Figure 1. Number of TBP, LBP and average birth weight of piglets according to three cluster

As result the sows of cluster two should be focused on, because only they reach acceptable values for number of piglets and birth weight. The number of litter did not differ between the sows of different cluster.

In the **second variant** of the first example 4 clusters have been chosen. The sows from cluster two in the first variant are divided into two groups. In table 2 the values of TBP, LBP, birth weight of piglets and number of litters are shown.

Table 2. Mean values and standard deviation of TBP, LBP, mean birth weight of piglets and number of litter of sows according to the cluster

parameter	cluster 1 (n = 91)	cluster 2 (n = 113)	cluster 3 (n = 81)	cluster 4 (n = 121)
TBP (MV±SD)	15,6 [a] ± 1,8	13,8 [b,c] ± 1,4	9,5 [b,d,f] ± 1,6	12,2 [b,d,e] ± 1,0
LBP (MV±SD)	15,0 [a] ± 1,6	13,2 [b,c] ± 1,2	9,1 [b,d,f] ± 1,6	11,8 [b,d,e] ± 1,0
average birth weight in kg	1,3 [a,d] ± 0,2	1,6 [b,c] ± 0,1	1,6 [b,f] ± 0,2	1,3 [d,e] ± 0,1
number of litter (MV±SD)	3,8 ± 2,3	4,1 [a] ± 2,2	3,2 ± 2,1	3,0 [b] ± 2,7

a:b; c:d; e:f $p<0,05$

Sows of the first cluster realize high numbers of TBP and LBP but their piglets do have a low average birth weight. Sows in cluster two (n=113) achieve comparable high numbers of piglets in combination with also high values for the average birth weight of piglets. They have 13.2 LBP with an average birth weight of 1.6kg. The piglets from the sows of the third cluster are the heaviest, but the number of piglets of those sows is the lowest. In the fourth cluster the sows achieve average results. In figure 2 the data are demonstrated.

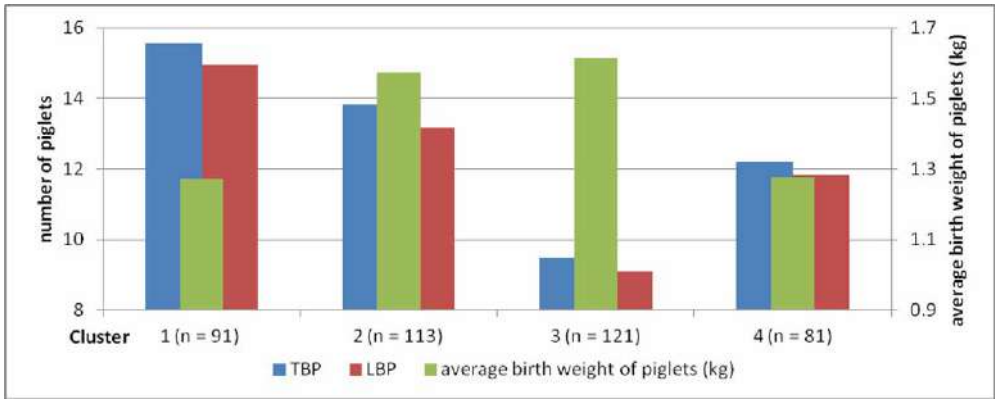

Figure 2: Numbers of TBP, LBP and average birth weight of piglets according to four clusters

It gets evident that sows of cluster two achieve results which are opposite to the expected trend. They are able to realize high numbers of piglets in combination with an appropriate birth weight. It is recommended to observe the following litters of those sows. Maybe they confirm those results. Then one should have a closer look on the genetics of those sows.

In a **second practical example** 1.802 sows of the 1st to the 12th litter have been clustered. For a more complex interpretation of the cluster five parameters have been chosen. These are the number of total born piglets (TBP) and live born piglets (LBP), boar, technician for insemination and number of litter. This example is mainly used to demonstrate the way of clustering. At first the relevance of the parameter and algorithms of fusion need to be approved. Therefore a table gets created which contains all seven algorithms of fusion. The sows get grouped in predefined three to five cluster. If there is a significant influence ($p \leq 0.05$) between a parameter and the group of a cluster a number 1 is given. This practice allows getting a first and closer look on the clustering from the statistical but not from the practical point of view. In table 3 a summary of those results of the test for significance is given in the form of sums. The maximum value for the method of fusion is 15, and the one for the parameter is 21. There can be at maximum a number of 35 significances for the number of cluster. The scales for distance and similarity have not been changed because of a loss of visibility.

Table 3. Summary of significances of the single parameter

parameter		method of fusion		number of cluster	
		1st method	15		
TBP	18	2nd method	15		
LBP	21	3rd method	9	3 cluster	30
boar	19	4th method	15	4 cluster	30
technician	16	5th method	7	5 cluster	31
number of litter	17	6th method	15		
		7th method	15		

It is clear that all five parameters have a significant influence on clustering. A closer look on the method of fusion shows, that method 3 and 5 are not suitable. The other methods are possible to use from the statistical point of view. The question which number of cluster would be the best is impossible to answer this way. The results do not differ enough. As a result of the test of significance all five parameter need to be analyzed with five methods of fusion and need to be interpreted for a number of three to five cluster. This means that 15

different results need to get interpreted with the help of mean values and cross tables in order to detect the model which fits best for biology and economy. By the way it is also possible that only one algorithm of fusion is useful because the results from the other methods do not show enough differences between the sows of a cluster.

As the result the best model is one with five clusters achieved with the complete linkage method. Means and standard deviations of the metric variables are shown in table 4. After that in figure three it is demonstrated very clear that sows of cluster three (n=337) do realize the best results with 17.5TBP and 16.3 LBP and an average number of litters of 2.2. Those sows should be examined further if they are able to use their high potential furthermore in the following litters. The sows in cluster four achieve very low results. They also need to be observed in the following litter, if they are able to realize better results.

Table 4. Mean value and standard deviation of the parameter TBP, LBP and number of litter according to the sows within the five

parameter	cluster 1 (n =1007)	cluster 2 (n = 156)	cluster 3 (n = 337)	cluster 4 (n = 152)	cluster 5 (n = 150)
TBP (MV±SD)	$14.91^{a} \pm 1.95$	$10.90^{b} \pm 1.68$	$17.49^{c} \pm 2.42$	$7.82^{d} \pm 2.23$	$12.61^{e} \pm 1.84$
LBP (MV±SD)	$13.66^{a} \pm 1.76$	$9.45^{b} \pm 1.77$	$16.33^{c} \pm 2.24$	$7.24^{d} \pm 2.06$	$11.59^{e} \pm 1.65$
Number of litter (MV±SD)	$4.11^{a} \pm 1.95$	$6.35^{b} \pm 1.66$	$2.20^{c,d} \pm 1.73$	$2.61^{c,d} \pm 1.68$	$1.30^{e} \pm 0.81$

a:b:c:d:e $p \leq 0.05$

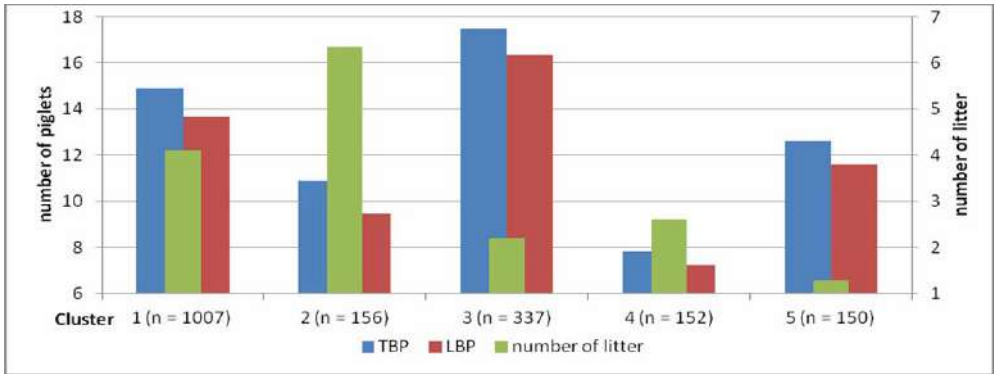

Figure 3. Number of TBP, LBP and number of litters according to the cluster

Boar and technician have been also recognized for clustering. Cross table have been used to identify differences between the clusters. For all inseminations six lines of boars have been used. It is noticeable no sow in cluster four was inseminated with a boar of line two. In cluster three no remarkable results could be detected. All boars have been used in the same amount. Differences are in the case of the technician for insemination. In total ten technicians are in the investigation. Sows of cluster one and two have been inseminated from the same five technicians in 99.2% of all cases for cluster one and 98.7% for cluster two. One additional technician inseminated 60.5% of all sows in cluster three and 86% in cluster five. The real influence of the technician needs to be evaluated. The reasons for the good results of the sows in cluster three need to get investigated furthermore.

Klaster analiza - statistički alat za analize složenih odnosa unutar različitih parametara kod svinja

H. Schlegel, K. Fischer, M. Waehner

Rezime

U savremenoj poljoprivrednoj proizvodnji analiziraju se brojni pokazatelji fertiliteta ili tovnih performansi svinja. Klaster analiza se koristi da bi se ispitao veći broj parametara istovremeno. Ovu analizu je moguće primeniti čak i kada se ne zna ništa o međusobnom odnosu ispitivanih parametara. U cilju demonstracije primene i obima klaster analize navedena su dva primera iz proizvodnje prasadi. U prvom primeru tri parametra su korišćena za optimalno grupisanje: broj ukupno rođene prasadi, broj živorođene prasadi i telesna masa prasadi na rođenju. Takođe, u drugoj varijanti ovog istog primera, dodat je i broj legala, kao još jedan parametar, da bi se pokazale razlike u interpretaciji u slučaju upotrebe različitog broja parametara. U drugom primeru prikazana je složenost klaster analize. U ovom slučaju upotrebljeno je pet parametara: broj ukupno rođene prasadi, broj živorođene prasadi, nerast, tehničar za osemenjavanje i broj legala. Tumačenje je direktno povezano sa brojem parametra. Ako se koristi veliki broj parametara neophodan je oprez u pravilnom postavljanju podataka za njihovo grupisanje.Vreme koje je neophodno ne bi trebalo da bude potcenjeno, pogotovo ako se ovaj metod poredi u odnosu na druge. Takođe, neophodan je oprez i u tumačenju rezultata, posebno ako se na osnovu njih donose odluke vezane za uzgoj. Generalno, klaster analiza je korisna alatka sa mnogo dodatnih mogućnosti za otkrivanje novih i složenih korelacija.

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THE EFFECTS OF RELATIONSHIP OF METABOLISABLE ENERGY, LYSINE AND THREONINE IN THE DIETS OF THE FIRST TWO THIRDS ON GESTATION OF SOWS

B. Živković[1], W. Migdal[2], G. Cilev[3], Č. Radović[1], M. Gogić[1], G. Marinkov[1], Lj. Stojanović[1]

[1]Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080, Belgrade-Zemun, Republic of Serbia
[2] Akademia Rolnicza, Kraków, Poljska
[3] University "St Kliment Ohridski" Veterinary Faculty, Bitola, Macedonia
Corresponding author: zivkovicbbranislav@yahoo.com
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Abstract: The effects of the use of ratios lysine:metabolisable energy and threonine:metabolisable energy in the nutrition of gestating sows during the first two thirds of gestating period were investigated. Experimental (the second group in the experiment) sows were fed during the first 76 days of gestation with experimental diet and till the end of gestation both groups of sows were fed with equal diets. All lactating sows were fed the same diets and all suckling piglets also were fed with creep fed during the lactation period. Research characterized was carried out total 21 sows in the two treatments (11 animals in the first, control group and 10 animals in the second, experimental group) were included in the experiment, on Experimental pig farm in Institute for Animal Husbandry, Zemun in Serbia. Obtained results showed that in the control, gestating diet characterized with lysine:ME 0.51 g/MJ and threonine:ME 0.40 g/MJ, were losing 17.70% of their weight during lactating period. During the same period the sows consumed 4.04 kg/day of food, 9.45 piglets/litter were weaned with 6.45 kg of average body mass at weaning. Average mass of whole litter in the first, control, group was 60.98 kg at weaning, which means that average daily gain of piglets was 169 grams. The feed intake in creep feeding was 5.80 kg/litter. The second, experimental, group of animals fed the gestating diet characterized with lysine:ME 0.61 g/MJ and threonine:ME 0.41 g/MJ sows in lactatation were losing 14.60% of their own body mass which was by 17.5% less compared to the control group of animals. Feed intake in experimental group of sows in lactation was 4.46 kg/animal, which was by 10,40% higher compared to the control group. With realized 9.60 piglets per litter at weaning and average body mass of 7.15 kg/animal, the litter weight of the experimental group was 68.51 kg which was by 12,34% better compared to the control group of animals. Average daily gain of experimental group of piglets was

187 grams i.e. the improvement by 10.65% and piglets consumed 5.90 kg of feed creep feeding, which was by 1.72% less of feed compared to the control group of piglets. Apparent coefficients of digestibility of dry and organic matter, protein and fibre showed that the level of utilization of all tested indicators was similar in both compared groups at the end of study period, i.e. first 70 days of gestation. Economical analysis of feed costs showed that the feeding in gestating sows and lactating ones the trial mixtures, as well as the diet in creep feeding, increased the cost of mixture by 1.53%. However, realized value of legal realized piglets at the end of lactation showed that the piglets of the control group have realized by 12,34% lower value of realized piglets compared to the animals of experimental group. In general, the obtained results showed that by correcting lysine:ME and threonine:ME ratios for the first two thirds of gestation, the sows have better production performance, the similar results concerning apparent digestibility of nutrients and the price of realized piglets are achieved, for lactating sows.

Keywords: Ratio lysine:ME, threonine:ME, the first two thirds of gestating sows, suckling piglets

Introduction

The 1998 National Research Council's (NRC) nutrient recommendations for swine suggested a fixed amount of dietary amino acids for sows during gestation, which assumes a constant demand for amino acids throughout gestation.

These recommendations were based on maintenance and growth data from the 1970s and 1980s. Since that time, considerable improvements have been made through genetic selection to indicate that the amino acid requirements for gestation are likely greater than current NRC recommendations.

During last 45 days of gestation, foetal weight increases five-fold, foetal protein increases 18 fold and mammary protein content increases 27 fold, indicated that the requirements for amino acids may be greater in late gestation compared with early gestation.

There threonine was the most likely amino acid to reflect the changing whole body amino acids requirements of sow in gestation.

Daily demand for threonine may change to a greater extent form early to late gestation than the requirement for lysine *Goihl J. (2011)*.

Our initial studies *(Živković et al., 2011)* showed that by correcting lysine:ME and threonine:ME ratios for early gestating, first third of the gestating sows, better production performance, digestibility of nutrients and the price of realized suckling piglets is achieved for lactating sows.

Objective this paper was to evaluate the use of ratios lysine:threonine in the nutrition of gestating sow during the first and second part. 1-76 feeding days, of gestating period were investigated.

Material and Methods

Tests were performed on experimental pig farm of the Institute for Animal Husbandry, Belgrade-Zemun. The experiment included a total of 21 sows divided into two dietary treatments. Since the beginning of pregnancy to 76th day of pregnancy, pregnant sows were successively introduced into the test, where sows were housed in group boxes up to 7 days before farrowing, and fed daily ration of 3.0 kg/head (Table 1). The first group was fed a mixture of standard composition with normal concentrations of lysine and threonine, 0.615 and 0.501, respectively.

Table 1. Scheme of the experiment

Group	1 control	2 experimental
Gestating sows, 1 – 76 days of gestation		
Control mixture, kg/days/head	3.0	-
Experimental mixture, kg/days/head	-	3.0
Gestating sows, till the end o gestating		
Control mixture, kg/days/head	3.0	3.0
Lactating sows, during whole lactating period		
Control mixture, kg/days/head	Ad libitum	Ad libitum
Suckling piglets – creep feeding		
Control mixture, kg/days/head	Ad libitum	Ad libitum

Sows of other experimental groups, groups received a meal of similar composition in which they are used increased levels of lysine (0.735) and threonine (0.510) in the mixture. Ratio lysine to ME was + 7.8% for threonine and ME reduced 7.9% in the experimental group compared to the control mixture (Table 2).

Table 2. Scheme of diets in gestating sows during the first 76 days of gestation

Group	1 control	2 experimental	%
ME, MJ/kg	12.04	13.13	+ 10.7
Lysine, total, %	0.615	0.735	+ 19.5
Threonine, total, %	0.501	0.510	+ 1.2
Lysine : ME	5.11	5.51	+ 7.8
Threonine : ME	4.16	3.83	- 7.9
Lysine : Threonine	100:81.5	100 :69.4	14.8

At 10 days before farrowing, sows were transferred to farrowing facility. Sows of the first - control and the second - experimental group were fed diets for lactating sows, with the same mixture used for lactating sows.

During lactation sows of both groups were fed *ad libitum* with individual feeding.

After farrowing, from the 10th day of age, piglets were fed diets – pre-starter mixture of same composition for both groups.

Around 70th day of gestation, in the diets for pregnant sows, $Cr_2O_3$was included in the food in order to examine and compared the digestibility of nutrients in diets using the indirect method.

The criteria for evaluation of the results were the following indicators: body weight of sows before farrowing and weaning, feed intake in lactating sows, the number of born piglets, number of piglets weaned, the average weight of pigs at farrowing and weaning, average daily gain of piglets and litter during lactation.

Statistical analysis of growth data and digestibility coefficients was performed by classical methods of statistical analysis, analysis of variance, and the data on differences between the average values using the t-test.

Results and Discussion

Period of gestation and lactation

The experiment examined the possibility of using a mixture of adequate improved mixtures in regard to lysine and threonine in the diet for pregnant sows during the first two thirds of pregnancy.

The results obtained (Table 3) showed that sows in the control group fed the normal farm mixture during lactation lost 17.70% of their own weight. Sows of the second - experimental, group, fed diet with the "narrower" threonine to ME ratio have lost only 14.60%, which is less by 17.51% in comparison with the ratios achieved with a mixture of control diet.

Table 3. Performance of sows in the experiment

Group	1 control	2 experimental
Lactating sows		
Body mass of sows at farrowing, kg	204.4	207.0
Body mass of sows at weaning, kg	217.6	191.3
Losses of body mass of sows, %	17.70	14.60
Compared to the control group, %	-	+ 17.51
Feed intake of sows, kg/days/head	4.04	4.46
Compared to the control group, %	-	+ 10.40

In terms of feed intake during lactation (Table 3) sows fed experimental mixture with the adjusted ratio of threonine to ME resulted in average daily feed intake of 4.46 kg. Use of the corrected ratio of threonine and ME in the mixture during pregnancy led to increased consumption, in average of 0.42 kg or 10.40% compared to the control mixture.

Table 4. Digestibility of nutrients of gestating sows in the experiment

Group	1 control	2 experimental
Gestating sows, at 70th days of gestation		
Dry matter, %	74.59	74.54
Organic matter, %	77.64	79.33
Crude protein, %	67.82	67.00
Fiber, %	45.93	48.53

On the70th day of gestation digestibility coefficients of studied indicators showed positive effects on organic matter and fibre in sows of the experimental group (Table 4).

Table 5. Performance of suckling piglets in the experiment

Group	1 control	2 experimental
Suckling piglets – creep feeding		
Duration of lactation period, days	30.3	30.5
Number of equalised piglets/litter	12.2	11.1
-average body weight of piglets at farrowing, kg	1.320[a]	1.428[a]
Compared to the control group, %	-	+ 7.42
Number of weaned piglets/litter	9,45	9,60
Compared to the control group, %	-	+ 1,59
Average body weight of piglets at weaning, kg	6,453	7,136
Average daily gain of suckling piglets, g	164	187
Compared to the control group, %	-	+ 10.65
Average body mass of litter at weaning. kg	60,98	68,51
Compared to the control group, %	-	+ 12.34
Consumed of pre-starter, kg	5,80	5,90
Compared to the control group, %	-	1,70

In terms of the number of live born piglets in experimental group, with 12.2 piglets born alive per litter, in average by 1.1 piglets or 9.02% more piglets were born than in the control group of sows. At the end of lactation, the use of the corrected ratio of threonine and ME led to 12.34% heavier weaned piglets by in

average 7.53 kg or 12:34% compared with the first - control group using the diet of usual composition (Table 5).

Significantly (P<0.05) higher body weight of piglets at farrowing were recorded in the experimental group by about 9.18%. Body weight of piglets in the experimental group, at weaning, by 23 g or 10.65% expressed positive impact on gain of piglets during lactation.

Economic analysis of the cost of the compared mixtures indicated the increased feed costs in gestating animals by 2.39%, in lactating sows difference was 1.28%, so that the recapitulation showed that the experimental diet in sows and piglets increased food costs by 1.53% compared the control diet mixture. The value of piglets realized in the experimental group shows that by 12.34% better realization of piglets is achieved which justifies the experimental diet for pregnant sows in the first two thirds of gestation, i.e. from 1-76 days of pregnancy (table 6).

Table 6. Economic analysis the cost of gain both sows and suckling piglets

Group	1 control	2 experimental
Gestating sows		
The price of feed for gestating sows 1 – 76 days of gestation	100.0	102.39
The price of whole gestation,	100,0	101,62
Lactating sows		
The price of feed whole lactating period, %	100,0	101,28
Suckling piglets		
The price of feed/litter at weaning, %	100,0	101,72
RECAPITULATION		
Cost of feed, sow + suckling piglets, %	100,0	101,53
The value of realised piglets/litter, %	100,0	112,34
Compared to the control group, %	-	+ 12,34

A group from Germany has recently published new recommendations for swine nutrition (*GfE, 2008*). These recommendations suggest that amino acid requirements in late gestation are greater than in early gestation. They (*GfE, 2008*) proposed a change of diet on day 85 of gestation to accommodate the greater amino acid requirements caused by increased foetal growth. For example *GfE (2008)* suggested standardized ileal digestible lysine intake of 9.4 g/d for day 1 to 85 of gestation and 14.6 g/d for day 85 – 115. The corresponding calculated values for threonine, based on estimated amino acid ratios for sow maintenance and body protein growth are 6.6 g/d and 9.6 g/d. These recommendations should be treated with caution until they are confirmed with experimental data.

There are a great deal more to be learned about energy and amino acid requirements during different phases of gestation and lactation. As this information becomes available, they expected to further reduce the cost of sow feeding while

maintaining of increasing sow productivity and longevity (*Moehn et al., 2009*). The above information has been to develop a revised feeding program for gestating sows:

- Lower energy and lower protein intake for early gestation from day 1 to 84.
- Higher energy and higher protein diet for late gestation from day 85 to 115.

There is potential to significantly reduce the cost of sow feeding, however, current nutrient recommendations need improvement. Phase feeding, using separate diets for early/mid gestation and late gestation, may save up to $5.00 per sow per gestation. Using a single diet for gestation, but phase feeding at a higher level in late gestation, may save up to $3.00 per sow per gestation. During late gestation and lactation, addition of free amino acids can reduce feed cost. These improvements may also increase sow productivity and increase sow longevity (*Moehn et al., 2009*).

Current amino acids recommendations for sows are to provide a fixed amount of amino acid intake throughout gestation based on the assumption that there is a constant demand for amino acid; however, the demand for nutrients changes from maternal lean tissue in early gestation to fetal and mammary growth in late gestation. The objective of this study was to determine the threonine requirement in early (day 35 to 53 and 25 to 55 for Exp. 1 and 2, respectively) and late (d 92 to 110 and 81 to 111) gestation using the indicator Amino Acids oxidation (IAAO) method as the tracer Amino Acid. Sows were used, each sow received each of 6 diets in random order in both early and late gestation. A basal diet was formulated to contain Threonine at 60% of the 1998 NRC recommendation and 20 and 60% of the 1998 NRC for early and late gestation, respectively. Crystalline l-Threonin was added to create additional diets with approximately 10% incremental increases in Threonine. Sows were placed in respiration chambers, and expired air and blood were collected every 30 min for 5.5 h. Tracer Phenilalnine was given orally over the last 4 h divided into eight 0.5 hours meals. Expired air and plasma were measured for 13CO2 enrichment and free Threonine concentration, respectively. Data were analyzed using a 2-phase nonlinear mixed model. The overall litter size and litter weight were 13.5 (3.1 and 20.5 3.9 kg), respectively. Based on IAAO, the threonine requirement in early gestation was 6.1 g/d . In late gestation, the threonine requirement based on IAAO was 13.6 g/d and 12.3 g/d). Based on plasma Threonine, the Threonine requirement in early gestation was 7.0 g/d and 3.9 g/d. In late gestation, the threonine requirement based on plasma Threonine was 10.5 g/d. There was a linear response to increasing threonine intake in late gestation in Exp. 1. Feeding a single amount of amino acids throughout gestation resulted in overfeeding amino acids in early gestation and under feeding amino acids in late gestation. The 2-fold increase in Threonine requirement in the last

third of gestation suggests that phase feeding sows in gestation will more closely meet the demands for nutrients and that the requirement for essential amino acids in gestating sows should be re-evaluated in early and late gestation separately (*Levesque et al., 2011*).

The researchers, *Ewan et al., (1999)*, weighed the sows at breeding, pre-farrowing, within 24 hours after farrowing and on days 7, 14 and 21 during lactation and weaning. They also adjusted litter sizes to at least 10 pigs per litter within three days of farrowing. Individual pigs were weighed at birth and on days 7, 14 and 21 days of lactation and weaning (*Ewan et al., 1999). Bell (2011)* state why it is necessary to pay attention to the energy, lysine and threonine needs in sows. The author notes that phase feeding regime of sows can save up to $ 12 per sow per year and thus increase its productivity.

The quality of produced milk and piglet growth were investigated in three groups of lactating Large White sows fed three mixtures containing different levels of lysine, threonine and crude protein. Piglets were weighed immediately after delivery and then in weekly intervals. At the age of 21 days the litter weight was the highest in group B (67.96 kg; $P < 0.05$), i.e. in the group with the highest dietary content of amino acids. An increased level of dietary amino acids became evident in milk at the first milk sampling (i.e. three days after delivery), when the content of lysine and threonine in milk was significantly the highest in group B (lysine 11.96 g/16 g N; threonine 6.72 g/16 g N; $P < 0.05$). The level of amino acids in milk and the differences between groups diminished in the following samplings. The changes of crude protein in milk were similar (*Bojčukova and Kratky, 2006*).

Beaulieu et al. (2008) mentioned that improved ileal digestibility of amino acid s in gestating sows is probably due to restricted feed intake and *Dourmad and Etienne (2002)* indicate that the optimal standardized digestible threonine:lysine ratio appears to be about 0.71 for multiparous gestating sows

Lysine requirements at two stages of gestation were estimated in adult sows who had been fed either low or high lysine diets in the previous lactation period. Sows fed low lost more weight during lactation that sows fed high levels. Also, sows fed low level diet had more days of anestrus than sows fed high levels. There were no differences, however, in litter weight gain of sows fed low levels and high levels. Estimates of gestation lysine requirements for sows fed low level were 9.6 and 12.2 g/d for early gestation and late gestation, respectively. The plasma urea nitrogen technique did not accurately or precisely estimate the lysine requirement of the sows fed the high level diet in lactation (*Sparks and Zimmerman, 2012*).

Conclusions

In general, the obtained results showed that the use of experimental mixture for gestating sows, from 1-76 days of gestation, based on ratios lysine and threonine versus ME can be recommended in the nutrition of gestating sows.

More research will be needed to establish threonin requirement for other essential amino acids and lysine other AA ratios separately for different phase of gestation.

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Uticaj odnosa metaboličke energije, lizina i treonina u ishrani prve dve trećine gestacije krmača

B. Živković, W. Migdal, G. Cilev, Č. Radović, M. Gogić, G. Marinkov, Lj. Stojanović

Rezime

Ispitivano je korišćenje odnosa lizina i ME i treonina i ME u ishrani suprasnih krmača tokom prve dve trećine suprasnosti. Ekspreimentalna, druga grupa krmača, je hranjena tokom prvih 76 dana eksperimentalnim obrokom a do kraja suprasnosti obe grupe krmača su hranjene istim obrocima. Sve krmače su tokom laktacije hranjene istim obrocima, a prasad na sisi su prihranjivana tokom laktacije istim smešama.

Istraživanja su izvedena na ukupno 21 krmači u dva tretmana (11 životinja u prvoj, kontrolnoj, grupi i 10 životinja u drugoj, eksperimentalnoj grupi je bilo uključeno u ogled koji je izveden na Eksperimntalnoj farmi svinja Instituta za stočarstvo, Zemun u Srbiji).

Dobijeni rezultati su pokazali da je kod kontrolnog obroka za suprasne krmače koji se karakterisao odnosom lizina i ME 0,51 g/MJ i treonina i ME 0,40 g/MJ su gubile od sopstvene težine 17,70% tokom laktacije. Tokom istog perioda krmače su konzumirale 4,04 kg hrane dnevno, zalučeno je 9,45 prasadi po leglu sa 6,45 kg prosečne telesne mase na zalučenju. Prosečna masa celog legla kod prve, kontrolne, grupe je bila 60,98 kg sa prosečnim dnevnim prirastom oko 169 grama. Konzumacija predstartera je bila 5,80 kg po leglu.

Kod druge, eksperimentalne, grupe prasadi od krmača koje su hranjene obrokom za suprasne krmače koji se karakterisao odnosom lizina i ME 0,61 g/MJ i treonina i ME 0,41 g/MJ tokom laktacije su krmače gubile 14,60% od sopstvene težine što je za 17,50% manje u odnosu na kontrolnu grupu životinja. Konzumacija hrane kod eksperimentalne grupe krmača tokom laktacije je bila 4,46 kg po hranidbenom danu koja je bila u proseku za 10,40% veće u poređenju sa kontrolnom grupom. Sa zalučenih 9,60 prasadi po leglu prosečne telesne mase 7,15 kg/prase, težinom celoga legla 68,51 kg što je bilo za 12,34% više u poređenju sa kontrolnom grupom životinja. Prosečan dnevni prirast kod eksperimentalne grupe prasadi je bio 187 grama što je poboljšanje za 10,65% i potrošnjom predstartera 5,90 kg hrane što bilo za 1,72% manje hrane no u prasadi kontrolne grupe.

Prividna svarljivost suve i organske materije, protein i celuloze su pokazali da je stepen iskorišćavanja svih ispitivanih parametara bio sličan kod obeju upoređivanih obroka na kraju ispitivanog perioda odnosno sedamdesetog dana suprasnosti.

Ekonomska analiza cene koštanja hrane je pokazala da se ishranom suprasnih i krmača u laktaciji, a takođe prihranjivanje prasadi povećava cena hrane za 1,53%. Ipak vrednost realizovane prasadi na kraju laktacije kod kontrolne grupe je bila za 12,34% manja u poređenju sa oglednom grupom prasadi.

U celini dobijeni rezultati su pokazali da se korekcijom odnosa lizina i treonina i ME u prve dve trećine gestacije krmača ostvaruje bolje proizvodnja, slično iskorišćavanje hranljivih materija iz smeša i povoljniji troškovi ishrane kod prasadi na sisi.

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THE EFFECT OF GENOTYPE, YEAR, AND FARM ON THE VARIABILITY OF TRAITS IN THE PERFORMANCE TEST OF GILTS

M. Gogić[1], M. Petrović[2], B. Živković[1], Č. Radović[1], D. Radojković[2], N. Stanišić[1], D. Stanojević[2]

[1]Institute for Animal Husbandry, Autoput 16, 11080, Belgrade-Zemun, Republic of Serbia
[2]Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080, Belgrade-Zemun, Republic of Serbia
Corresponding author: gogic.marija@gmail.com
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Abstract: The aim of this study was to determine the effect of genotype of gilts, the test year and farm on performance of tested gilts. Traits that were examined were age at the end of the test (AET), lifetime daily gain (LDG), fat thickness 1 (FT1), fat thickness 2 (FT2), depth of dorsal muscle (MLD) and estimated lean meat content (LMC). In the present research the four farms in Central Serbia were analyzed in three consecutive years, with a total of 6497 gilts tested. Gilts of Swedish Landrace breed (SL) and F1 generation between Swedish Landrace and Large White (SL × LW, where the mother of Swedish Landrace and Large White breed father) were studied. The results show that year of testing of gilts and farm had statistically significant effect on the expression of all traits ($P < 0.01$ and $P < 0.001$), while the genotype of gilts showed the statistical significance on the following traits: AET, LDG and FT2 ($P < 0.01$ and $P < 0.001$) and no statistically significant effect on the properties of FT1, MLD and LMC ($P > 0.05$)**.**

Key words: genotype, gilt, year, farm, the performance test

Introduction

Performance test is a basic tool for the study of offspring, candidates for further selection. Selecting gilts is performed based on the results of performance (direct) test or own production traits that can be measured on individual animals. Considering that performance test covers medium to high heritable traits, it can be expected to improve these properties in the progeny generation. In Serbia, performance gilt test is carried out on the farm, where the group test is performed in a separate building. Gilts complete the test with 100 ± 10 kg. At the end of the

test, body mass (WET), fat thickness 1 and 2 (FT1 and FT2), depth of dorsal muscle (MLD) are measured, lean meat content (LMC) estimated and the lifetime daily gain (LDG) calculated.

Breeding value of animal does not depend on one but from more quantitative traits on which great influence have external factors. Investigations of many authors show that numerous fixed (year and season of birth, age, sex, nutrition and race) and random (individual effect of animal, sire and dam) factors influenced on breeding value of animals *(Brkić et al., 2001)*. Breeding value of pigs is determined, among others, from performance test results. It is well known that selection yields positive results if it includes genetic parameters that accurately reflect the value of the population being improved *(Szyndler-Nędza et al., 2010)*. Improvement of performance traits of pigs is obtained by applying right evaluation methods and in use of their results in conducted selection. One of them is performance test, which shows breeding value of pigs regarding to growth and slaughter performances *(Michalska et al., 2008)*. Based on the results of the test performed the next generation of parents is selected. Parents of the next generation should be chosen based on several properties, and if we take into account the information about ancestors and relatives then the accuracy of the breeding values is higher *(Petrović, 1991)*. The problem of producing quality breeding pigs has become more complex in recent decades as a result of, among other factors, a significant increase in the leanness of modern species and hybrids. The main criteria in the selection of gilts is a high gain, the minimum thickness of back fat and high lean meat content *(Kovčin et al., 2006)*. High numbers of total and especially live-born piglets are the basic requirement for an effective piglet production *(Fischer et al., 2009)*. One of the primary factors influencing the level of reproductive efficiency of the breeding herd is the number of quality pregnant gilts, introduced in the breeding herd for overhaul of sows culled from further reproduction *(Radović et al., 2008a, Radović et al., 2007, Radović et al., 2008b, Stančić et al., 2008)*. For this reason, gilts should have a greater fat thickness in order to have a sufficient supply of nutrients to be used during pregnancy and their growth. Productivity in terms of economically important traits, including the performance test traits of different breeds and lines of pigs reared in particular countries and regions is diversed and changes over the years *(Nowachowicz et al. 2011)*. Intensity of growth, food utilization and meat yield are of great importance in breeding and selection. Considering that quantitative traits and their expression are under the influence of several genes, they are under strong influence of environment factors. This shows the significance of accurate and as precise possible assessment of these traits, as well as of the breeding value of the animal *(Radović et al., 2013)*.The effects of test year and birth year were established ($P<0,001$) for all studied traits, whereas the effect of genotype was not established

(P>0.05) on traits of performance tested gilts *(Radović et al., 2012)*. The production features of performance tested gilts vary under the influence of their sires, the testing season and genotype of gilts *(Petrović et al., 1991, Petrović et al., 1999)*, and farms *(Gogić et al., 2012)*.

Material and Methods

The performance test included gilts from four farms in three consecutive years. Gilts tested belong to the following genotypes: The SL and SL × LW. A total of 6497 animals were tested, of which 2179 in the first year, 2089 in the second and 2229 in the third year. The first farm tested 1791 animals, the second farm 2448, the third farm 416 and the fourth farm 1842 animals. Distribution of the genotypes of gilts showed that 3754 Swedish Landrace gilts were tested, and 2743 crossbred animals. At the end of the performance test, the body weight of all animals was measured (WET), and using the 105 PIGLOG ultrasound, fat thickness 1 (FT1), fat thickness 2 (FT2), depth of dorsal muscle (MLD) were measured and lean meat content estimated (LMC). The PIGLOG 105 consists of a microcomputer and probes. The device can store values of about 900 animals, and then connects to the PC and thus the measured values are transferred to the PC. Measurements were made at the following locations: 1) FT1 between 3rd and 4th lumbar vertebra, 7 cm lateral to the back line; 2) between FT2 between 3rd and 4th back rib, 7 cm lateral to the back line; and 3) in the dorsal part of the MLD between 3rd and 4th back rib, 7 cm lateral to the back line. Studied traits in this study were following: age at the end of the test (AET), lifetime daily gain (LDG), fat thickness 1 (FT1), fat thickness 2 (FT2), depth of dorsal muscle (MLD), and estimated lean meat content (LMC).

Data analysis was performed using an appropriate computer program, using the method of Least Squares procedures *(LSMLMW and MIXMDL-Harvey, 1990)* to determine significance (P <0.05) of systematic influences on the properties of age at the end of the test, the life daily gain, back fat thickness 1 and 2, depth of back muscle and the estimated lean meat content. The model included: genotype of gilts, years of testing and farm. We used the following model:

$Y_{ijkl} = \mu + G_i + Y_j + F_k + b_1 (X_1 - \bar{x}_1) + \varepsilon_{ijkl}$

where: Y_{ijkl} = expression of the trait , i genotype, j year of testing, k farm, l gilt, μ = general population average, G = animal genotype, Y=year of testing, F = farm, b_1 = linear regression effect of body weight at the end of test, ε = random error, i = subscript for animal genotype (i =1, 2), j = subscript for year of testing (j =1, 2, 3), k= subscript for farm (k=1, 2, 3, 4), l = subscript for gilt.

Results and Discussion

Traits examined in this study were adjusted to the same body weight at the end of the test of 111.3 kg. Mean values with standard deviations of corrected traits are shown in Table 1.

Table 1. The average values and variability of the traits

Trait*		$\bar{x}$ ± SD	μ ± S.E
AET	Age at the end of the test, days	229,97 ±28,76	229,67 ± 0,28
LDG	Life daily gain, g	484,42 ± 59,17	485,01 ± 0,58
FT1	Fat thickness 1, mm	12,98 ± 3,17	13,26 ± 0,04
FT2	Fat thickness 2, mm	11,27 ±3,00	11,46 ± 0,04
MLD	Depth of dorsal muscle, mm	50,72 ± 5,76	50,56 ± 0,08
LMC	Estimated lean meat content, %	58,80 ± 2,90	58,52 ± 0,04

*Traits corrected for the same body weight at the end of the test 111,3kg

Table 2 shows the LSM ± SE values of the studied traits of gilts by source of variation: genotype, age, and farm.

Table 2.Average and standard deviation of tested traits according to sources of variation

Source of variation		AET[1)], days	LDG, g	FT1, mm	FT2, mm	MLD, mm	LMC, %
Genotype	SL	231,14±0,36	482,05±0,74	13,22±0,05	11,38±0,05	50,49±0,10	58,58±0,05
	SL×LW	228,20±0,37	487,98±0,76	13,30±0,06	11,55±0,05	50,62±0,11	58,45±0,05
Year	1	230,27±0,44	484,50±0,90	13,51±0,07	11,57±0,06	51,24±0,13	58,44±0,06
	2	230,54±0,43	483,42±0,88	13,55±0,07	11,91±0,06	50,34±0,12	58,11±0,06
	3	228,19±0,41	487,12±0,83	12,73±0,06	10,90±0,06	50,09±0,12	59,00±0,06
Farm	1	226,84±0,48	487,74±0,97	13,61±0,07	11,43±0,07	52,10±0,14	58,61±0,07
	2	251,90±0,41	438,11±0,83	12,47±0,06	11,52±0,06	52,11±0,12	59,09±0,06
	3	238,80±0,89	465,75±1,83	14,44±0,14	12,26±0,13	50,38±0,26	57,58±0,13
	4	201,15±0,43	548,44±0,87	12,82±0,07	10,64±0,06	47,63±0,12	58,79±0,06
WET (b)		0,606[2)***]	3,151***	0,179***	0,159***	0,153***	-0,144***

[1)] AET- Age at the end of the test; ŽDP-life daily gain; FT1- Fat thickness 1; FT2- Fat thickness 2; MLD- Depth of dorsal muscle, LMC- Estimated lean meat content; [2)] ***=P<0.001

Taking genotype as a source of trait variation, Table 2 shows that the Swedish Landrace sows were on average older than crossbreds by 3 days; animals tested in the second year were the oldest (230.54 days), while the animals on the

farm 2 were the oldest (251.9 days) and animals on farm 4 the youngest (201 day). Life daily gain was higher in gilts genotype SL × LW compared to purebred gilts by 5 g, but the crosses were younger; animals tested in the third year had the highest weight gain, while animals from farm 4 showed the highest weight gain (548.44 g), which is explained by the fact that these animals were also the youngest. In regard to the trait fat thickness, there were no major differences in the genotype, but animals in the third year of testing had the thinnest back fat and animals from the farm 3 had the thickest back fat. It was found to be almost no difference in the traits of MLD per genotype, but animals tested in the third year had the lowest depth of back muscle, and animals from farm 4 had significantly lower muscle depth, compared to animals from other farms. Also,in regard to the trait of leanness no significant differences in genotype were established, but there were differences by age and farms. The most lean were animals in the third year of testing, and had a value similar to the animals of the second farm which were the most lean (59%). It can be seen that the regression effect of the weight at the end of the test had a statistically significant influence on the manifestation of all the traits ($P < 0.001$). By increasing WET for one kilogram, an increase in AET by 0.6 days was achieved, LDG increased by 3.15 g, FT1 increased by 0.18 mm, FT2 increased by 0.16 mm, MLD increased to 0.15 mm while the lean meat content reduced by 0.14%.

According to *Vidović et al. (2009)* the body wight of gilts at the end of the test were varied by years of testing of 101-102 kg, while the average age of gilts genotype Swedish Landrace and crossbreds was 182 days, so these values are much lower of the same traits of our study.

Compared to results by *Gogić et al. (2012),* significant differences between genotypes of gilts can be observed, because the animals were younger, with higher LDG, thinner back fat and larger MLD compared to this research. Genotype of animals had no statistically significant effect on the traits of gilts *(Radović et al., 2003)*, but gilts were 236 days of age, with an estimated lean meat content/meat yield of about 42%.

When observing according to the years of testing an increase of the trait of LDG occured, which is consistent with the research of a number of researchers *(Čechova and Tvrdon, 2002; Petrović et al., 2009; Gogić et al., 2012)*, and *Radović et al. (2012)* have suggest that there is a decrease in the LDG trait when observed according to the years of testing.

Very similar results were obtained in studies by *Škorput et al. (2009)* for the trait FT2: in genotype SL it was 11.3 mm, and in F_1 crosses 11.9 mm, but animals were significantly younger when completed the test, 193 or 194 days of age.

Radović et al. (2012) have reported the value of the trait LMC in Swedish Landrace gilts of 54.89%, and 53.06 mm for MLD, whereas in our study the value of the LMC was 58.58% and MLD 50.49 mm.

In the research by *Gogić et al. (2012),* regression influence of WET on the expression of traits was statistically significant for all traits except for MLD where there was no significance, with negative sign for the traits of FT1 and FT2, while in the present study, WET had an impact on all the traits and the negative sign only occured in LMC feature.

Table 3 shows the level of significance of effects included in the model on the studied traits of gilts.

Table 3. Statistical significance (level of significance) of effects included in the model on studied traits

Variation source (effect)		AET[1)]	LDG	FT1	FT2	MLD	LMC
Model	Genotype	***[2)]	***	NS	**	NS	NS
	Year	***	**	***	***	***	***
	Farm	***	***	***	***	***	***
	R^2	0,612	0,618	0,193	0,250	0,179	0,176

[1)] AET- Age at the end of the test; LDG-life daily gain; FT1- Fat thickness 1; FT2- Fat thickness 2; MLD- Depth of dorsal muscle, LMC- Estimated lean meat content; [2)]NS=P>0,05; **=P<0,01; ***=P<0,001

By using this model the following results were obtained: 1) genotype of gilts showed statistically significance in respect to the traits of AET, LDG and FT2 (P <0.01 and P <0.001), but no effect on the properties of FT1, MLD and LMC (P> 0.05); 2) years of testing significantly affected the expression of all the traits (P <0.01 and P <0.001), 3) farm as a source of variation was also a statistically significant effect on the expression of all traits in performance testing of gilts (P <0.001). The coefficient of determination R^2 showed that the effects included in the model explained variation of AET with 61.2%, variation of LDG with 61.8%, variation of FT1with 19.3%, of FT2 with 25.0%, the variation of MLD with 17.9% and variation of LMC with 17.6%.

The influence of the year of testing on variations in the properties with statistical significance is consistent with research of *Radović et al. (2012).* Genotype in this study did not demonstrate the impact only on features FT1, MLD and LMC, while in the study by *Radović et al. (2012)* it failed to show any effect on any trait in the performance testing of gilts. In the research by *Gogić et al. (2012),* year of testing has shown a high statistical significance on the expression of all traits in line with our results, it also applied to the farm, however, only the farm

showed no effect on MLD trait in studies from year 2012. The production traits of performance tested gilts vary under the influence of year *(Petrović et al., 1991, 1995)*, which is consistent with our research. Also, *Nowachowicz et al. (2009)* have reported statistically significant effect on the properties of FT2 and MLD (P <0.001).

Conclusion

Based on the results of this study it can be concluded that the genotype of gilts showed a statistically highly significant effect (P <0.01 and P <0.001) on variation of properties AET, LDG and FT2, but did not show statistical significance (P> 0.05) on the traits FT1, MLD and LMC. Year of testing as a source of variation in traits showed statistically significant effect on the expression of all traits in performance testing of gilts (P <0.01 and P <0.001). Also, farm showed statistically significant effect on the variation of the traits (P <0.001). It was recorded, observed by years, that the increase in the value of the LDG, reduced the fat thickness FT1 and FT2, and at the same time increased the estimated lean meat content in carcass sides. Younger animals have a greater value for trait of LDG compared to older animals, and also had a thinner back fat and higher estimated lean meat content. In future breeding of gilts, the recommendation is not to focus too much on back fat thickness reduction, so that animals could normally reach sexual maturity and successfully present the gestation and raise the first litter.

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Uticaj genotipa, godine i farme na varijabilnost osobina u performans testu nazimica

M. Gogić, M. Petrović, B. Živković, Č. Radović, D. Radojković, N. Stanišić, D. Stanojević

Rezime

Cilj ovog istraživanja je da se utvrdi uticaj genotipa nazimica, godine testiranja i farme na osobine performans testiranih nazimica. Osobine koje su

ispitivane su: uzrast na kraju testa (AET), životni dnevni prirast (ŽDP), debljina slanine 1 (FT1), debljina slanine 2 (FT2), dubina leđnog mišića (MLD) i procenjena mesnatost (LMC). U ovom istraživanju su analizirane četiri farme na teritoriji Centralne Srbije u tri uzastopne godine, pri čemu je ukupno testirano 6497 nazimica. Ispitivane su nazimice švedskog landrasa (ŠL) i melezi F_1 generacije između švedskog landrasa i velikog jorkšira (ŠL×LW, pri čemu je majka rase švedski landras a otac rase veliki jorkšir). Rezultati istraživanja pokazuju da godina testiranja nazimica i farma imaju statistički značajan uticaj na ispoljavanje svih osobina ($P<0,01$ i $P<0,001$), dok genotip nazimica pokazaje statistički značaj na osobine AET, ŽDP i FT2 ($P<0,01$ i $P<0,001$) a nema statistički značajnog uticaja na osobine FT1, MLD i LMC ($P>0,05$).

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INFLUENCE OF STOCKING DENSITY ON CARCASS QUALITY OF BROILERS

A. Softić, A. Kavazović, V. Katica, V. Šakić, A. Katica, N. Mlaćo, M. Varatanović

Veterinary Faculty University of Sarajevo, 71000 Sarajevo, Bosnia and Herzegovina
Corresponding author: almira.softic@vfs.unsa.ba
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Abstract: The aim of this study was to determine the effect of stocking density on the portion of main parts of the carcass of broiler chicken provenience Cobb. The experiment was carried out on 80 one day old broilers of Cobb provenience divided into two groups (control and experimental) for up to 42 days of age. All chickens selected for the experiment were healthy and vital, and vaccinated according to an established program of immunization. Housing conditions (temperature, humidity, light program) were according to recommendations of the selection expert. Chickens of the experimental group represented a group of low stocking density (12 chickens/m^2), while the stocking density of control group was under the technological recommendations (15 chickens/m^2). At the end of the fattening period by a random sample there were selected and characterized 7 males and 7 females from each group and their final body mass was weighed. After exenteration and cooling in the carcasses processed in a way "prepared for the grill", the weight of the body, the proportion of the body in relation to the final weight were determined, as well as shares of the breast meat, drumsticks with thigh and abdominal fat in ratio to final body weight and carcass weight. These results indicate that the broiler chickens of control group separated by gender obtained better production results (final body weight, carcass weight, dressing percentage) compared to the experimental group of chickens, but the differences were not statistically significant. In addition, a higher, but not statistically significantly higher proportion of examined edible parts in final body weight and carcass weight, was found in male and female chickens obtained from control group that were stocking density was in accordance with technological recommendations.

Key words: stocking density, broiler chickens, carcass, quality

Introduction

Production of quality broiler meat is profitable and dynamic branch of animal husbandry. Compared to other livestock sectors, the prosperity of intensive poultry production, to a great extend depends on choice of the best commercial hybrids, condition of housing, diet and health care of poultry. In addition to these factors and related to way of keeping with goal of fattening, the stocking density of specimens per unit area of one square meter is more important environmental factor. Numerous studies have focused on the study of optimal conditions of housing in terms of production indicators, well-being and behaviour of specimens as well as the carcass quality *(Škrbić et al., 2009; Thomas et al., 2004)*.

Basic characteristics of intensive chicken meat production are mass use of hybrid specimens of high production potential, located in confined space, in order to achieve greater production of quality meat in shortest possible time, with minimum losses and with minimal food consumption. According to above mentioned, very important feature of broilers is a good carcass conformation and good quality meat. Carcass quality of broilers is mostly determined inadequate term and has a genetic and therefore the great economic significance *(Pavlovski and Mašić, 1983; Pavlovski et al., 2006)*, and it is estimated by yield and quality of edible parts of carcass *(Nikolova et al., 2008)*. These parts, yield and quality of some parts of carcass are affected by provenance, gender, age, health, diet, body weight, carcass rating and the time of the cease of feeding prior to slaughter *(Bouwkamp et al., 1973; Moran, 1977; Siegel, 1984)*. Besides that, great importance is also ready-meat chicken meat on the market and customer demand for these products.

The aim of this study was to determine the effect of stocking density on the portion of main parts of the carcass of broiler chicken provenience Cobb.

Materials and Methods

The experiment was carried out on 80 one day old broilers of Cobb provenience divided into two groups (control and experimental) for up to 42 days of age. Chickens from each group at immigration were homogeneous with respect to body weight. Housing conditions (temperature, humidity, light program) were according to recommendations of selection expert, but adapted to the experimental conditions hold. During the experiment food and water chicken were given *adlibitum* and vaccinated according to an established program of immunization against Newcastle and Gumboro disease.

Chickens of the experimental group represented a group of low stocking density (12 chickens/m^2), while the stocking density of control group was under the technological recommendations (15 chickens/m^2). At the end of the fattening period by a random sample there were selected and characterized 7 males and 7 females from each group and their final body mass was weighed. After exenteration and cooling in the carcasses processed in a way "prepared for the grill", the weight of the body, the proportion of the body in relation to the final weight were determined, as well as shares of the breast meat, drumsticks with thigh and abdominal fat in ratio to final body weight and carcass weight. Statistical analysis was performed using the statistical package computer program Microsoft Excel 2000, and the differences in the mean values between groups were tested by t test.

Results and Discussion

At the end of 42 days fattening, chicks of the control group achieved a higher average body weight, carcass weight and abdominal fat content compared to experimental group (Table 1). The average body weight of controlled group chickens was 2.385,81 g male and 2.257,13 g for females, which is 1,23% or 0,49% more compared to male and female chicken from experimental group, respectively.

Table 1. Body weight before slaughter, carcass weight and abdominal fat in investigated broiler chickens

Parameter, g	Experimental group	
	Male	Female
Body weight before slaughter	2.356,31 ± 68,78	2.246,17 ± 64,58
Carcass weight	1.651,33 ± 75,03	1.581,50 ± 58,55
Abdominal fat	17,38 ± 1,31	16,24 ± 1,17
	Control group	
	Male	Female
Body weight before slaughter	2.385,81 ± 105,12	2.257,13 ± 36,75
Carcass weight	1.703,51 ± 54,61	1.614,03 ± 42,66
Abdominal fat	18,74 ± 1,53	17,51 ± 1,37

Value expressed as x ±SE

According to offal values, determined average weight of carcass in males and females in the control group is 1.703,51 g, and 1.614,03 g, respectively. In the

experimental group the average weight of carcass in male chickens was 1.651,33 g, and 1.581,50 g in females, which is 3,06% and 2,01% less compared to the controlled group. Higher values of dressing percentage (Figure 1) were determined in chicken of control group of both sexes.

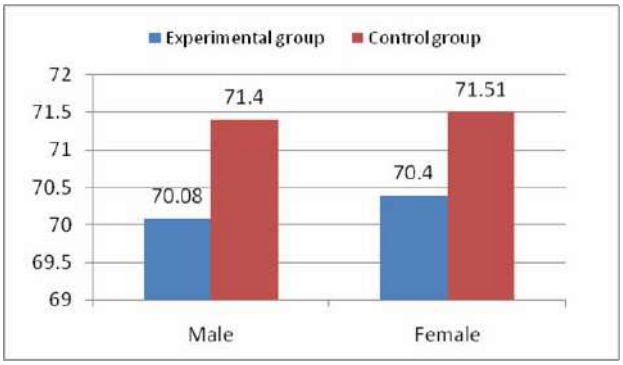

Figure 1. Dressing percentage (%) of chickens after 42 days of fattening

Comparing the impact of hybrid chickens Cobb 500 and Hubbard Classic, and gender on carcass quality, nutritional and technological properties of meat, *Kralik et al. (2011)* determined the body weight before slaughter and it was 2.085 g for males and 1.959 g for females, while the carcass weight was 1.478 g and 1.367 g, respectively, which is lower compared to results obtained in our study for both male and female chickens of both groups. *Nikolova et al. (2008)* also compared the effects of two genotypes (Cobb 500 and Hubbard Classic), gender of broilers and different composition of food (energy-protein) on the quality of broiler carcasses and found that the carcasses of cockerel were significantly ($P<0,05$) heavier than the carcass of female chicken. The results of our study indicate that both males and females of both groups had a greater carcass weight compared to the weight of chickens „prepared for the grill“obtained from the study *Nikolova et al. (2008)*. Actual carcass weight of males and females in our study did not differ significantly.

In our research, male chickens of the controlled group achieved higher but not significantly higher average body weight at the end of the fattening 42-days period, this is consistent with the results of *Softić et al. (2006)* which examined the impact of long fattening period and gender on the conformation and dressing percentage of broilers. *Gornowicz et al. (2009)* in their research in stocking density of 14,5 chicken/m^2 found that genotype (Cobb 500, Hybro G, Ross 308) had no significant effect on the final body weight of chickens, as opposed to gender where significant difference was determined which is not in compliance to our results.

From the Graph. 1 we can see that the determined dressing percentage is approximately the same in chickens in the control and experimental group which is in alliance with the results of other authors *(Kralik et al., 2007; Nikolova et al., 2008)*.

Škrbić et al. (2008) found that at lower stocking densities (12 chicken/m^2) of Cobb broilers significantly ($p<0,01$) increased is a final body mass for both genders as well as the mass of carcass „traditional processing“, „ready for roasting“ and „ready to grill“, which is not in accordance with the results of our study where a higher final weight of chickens of controlled group was found (density 15 chicken/m^2). Our results do not confirm the statistical validity of the impact of stocking density on the average body weight which is consistent with the results of *(Thomas et al., 2004; Gornowicz et al., 2009)*, as well as dressing percentage *(Škrbić et al., 2008; Thomas et al., 2004)*. Looking at the final body weight, carcass weight and both genders dressing percentage in accordance to technological recommendation (15 chicken/m^2), has achieves slightly better results than the experimental group (12 chicken/m^2), and the reason is probably larger living space which resulted in irrational use of energy, they moved more, or at least had more possibilities for moving, did not use food rationally and so they achieved lower indicators. In this context our results are matching to the results of *Softić, (2005)* where is stated that with full respect of production technology it is possible to achieve better production results.

Table 2 shows the weight of main parts in carcass, and the Table 3 shows portions breasts, drumsticks with thigh and abdominal fat in relation to the average live weight and carcass weight.

Table 2. The weight of main parts in carcass

Part of carcass	Weight of main parts in chicken carcass (g)			
	Experimental group		Control group	
	Male	Female	Male	Female
Breast	509,48± 26,89	488,69± 19,78	527,47± 15,51	492,56± 16,36
Drumstucks with thigh	372,87± 19,43	340,17± 14,09	385,20± 15,57	350,43± 15,66
Abdominal fat	17,39± 1,31	16,24± 1,17	18,74 ±1,53	17,51± 1,37

Value expressed as x ±SE

Table 3. Shares of breasts, drumsticks with thigh and abdominal fat in relation to the average live weight and carcass weight

Part of carcass	Portions of main parts in carcass in relation to the average live weight (%)				Portions of main parts in carcass in relation to the weight of the carcass (%)			
	Experimental group		Control group		Experimental group		Control group	
	Male	Female	Male	Female	Male	Female	Male	Female
Breast	21,62	21,76	22,10	22,26	30,85	30,90	30,96	31,14
Drumsticks with thigh	15,82	15,14	16,14	15,52	22,58	21,51	22,61	21,71
Abdominal fat	0,74	0,72	0,78	0,77	1,05	1,02	1,10	1,08

Proportion of breasts, drumstick with thigh and abdominal fat compared to the average live mass do not differ significantly among broilers in relation to gender and applied population density *(Škrbić et al., 2008)*, but it can be concluded that their values were higher in controlled group (15chicken/m^2). In terms of gender, regardless of stocking density it can be concluded that the proportion of breasts compared to the average live weight was higher in female chicken which is consistent to the results of *Škrbić et al. (2008); Škrbić et al. (2009)*, although our results obtained in relation to the aforementioned authors are slightly higher. Our results indicate higher values of the breast in relation to the live weight from the results of *Nikolova et al. (2008); Nikolova and Pavlovski (2009); Alibegović-Zečić et al. (2003)*, and higher values of proportions of drumstick with thigh compared to the average live weight and carcass weight *(Alibegović-Zečić et al., 2003)*, noting that the mentioned authors through their work have nominated different experimental designs.

Conclusions

Based on research results of carcass quality of broilers Cobb, depending on stocking density, the following conclusions can be drawn:

1. Control group chickens separated by gender have achieved better production results (final body weight, carcass weight, and yield) compared to the experimental group of chickens, although the differences were not statistically significant.
2. Higher, but not statistically significantly higher proportion on edible parts in relation to the final weight and carcass weight was determined in male and female chickens whose population was in accordance with technological standards of the breeder.

Uticaj gustoće naseljenosti na kvalitet trupa brojlerskih pilića

A. Softić, A. Kavazović, V. Katica, V. Šakić, A. Katica, N. Mlaćo, M. Varatanović

Rezime

Cilj istraživanja bio je utvrditi uticaj gustine naseljenosti na udeo pojedinih delova trupa brojlerskih pilića provenijence Cobb. Ogled je proveden na 80 jednodnevnih tovnih pilića provenijence Cobb podeljenih u dve grupe (kontrolna i ogledna) u trajanju do 42 dana. Svi pilići odabrani za ogled bili su zdravi i vitalni, te vakcinisani prema utvrđenom programu imunoprofilakse. Uslovi smeštaja (temperatura, vlaga, svetlosni program) bili su u skladu sa preporukama selekcionera. Oglednu grupu su predstavljali pilići sa manjom gustoinom naseljenosti (12 pilića/m^2), dok je naseljenost kontrolne grupe bila u skladu sa tehnološkim preporukama (15 pilića/m^2). Na kraju perioda tova metodom slučajnog uzorka odabrano i obeleženo je po 7 muških i 7 ženskih jedinki iz svake grupe i izmerena završna telesna masa. Potom nakon egzenteracije i hlađenja u obrađenim trupovima obrađeno na način „pripremljeno za roštilj" određena je masa trupa, udeo trupa u odnosu na završnu telesnu masu, te udeli grudnog mesa, bataka s karabatakom i abdominalne masti u odnosu na završnu telesnu masu i masu trupa.

Dobijeni rezultati pokazuju da su jedinke kontrolne grupe odvojene po polovima ostvarile bolje proizvodne rezultate (završna telesna masa, masa trupa, randman) u odnosu na oglednu grupu pilića, ali utvrđene razlike nisu bile statistički značajne. Pored toga, veći, ali ne i statistički značajno veći udeo ispitivanih jestivih dielova u odnosu na završnu telesnu masu i masu trupa utvrđen je kod muških i ženskih pilića čija je naseljenost bila u skladu sa tehnološkim normativima selekcionera.

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THE IMPACT OF PHYTOGENIC ADDITIVES ADDED INTO DIET ON ECONOMIC RESULTS OF BROILERS PRODUCTION

Đ. Okanović, R. Čolović, D. Milić, N. Vukelić, V. Zekić, T. Tasić, P. Ikonić

[1]University of Novi Sad, Institute of Food Technology, Bulevar cara Lazara1, 21000 Novi Sad, Serbia
[2]University of Novi Sad, Faculty of Agriculture, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia
[3]Perutnina Ptuj – Topiko AD, Petefi Brigade 2, 24300 Bačka Topola, Serbia
Corresponding author: djordje.okanovic@fins.uns.ac.rs
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Abstract: Production of broiler practically presents the most intensive branch of the animal husbandry. The reproduction process is relatively short, which enables faster turnover of the engaged resources. The influence of phytogenic additives addition in broiler diet on the economic results of the production were examined in present paper. The experiment was conducted on broilers hybrid ROSS (n = 16480) divided in two groups. First group (control, C) was fed with commercial broiler feed mixture, while in experimental group (E1) phytogenic additives were included, respectively. Fattening of broilers lasted for 40 days. Food and water were provided *ad libitum* in the floor fattening system. Research of the economic results of the production of broilers is based on the determination of the total production cost, value of the production and the financial result. Calculation of these economic categories is based on the data gathered on the selected farm. It was concluded that the diet enriched with phyitogenic additives had beneficial effect on the majority of monitored economic parameters: total benefit (2 353,97 : 1 870,30 €), benefit per broiler (0.29 : 0.23 €), economy (1.09 : 1.08) and profitability (3.27 : 2.61%).

Key words: broilers production, phytogenic additives, cost

Introduction

Animal husbandry presents the most intensive branch of agriculture and has multiple significances, for both producers and consumers. The increase in the production of the meat, milk, eggs, among others is the foundation for the improvement of the nutrition structure of the population with highly valuable animal proteins *(Tica et al., 2009; Cmiljanić et al., 2005)*.

Broilers fattening, as a final phase in the production line of chicken meat within the modern intensive poultry production based on industrial principles, is the fastest and the most rational way of producing poultry meat *(Okanović et al., 2011)*.

In a floor system and on a deep mat, mainly heavy type proveniences are fattened, that are characterized by intensive growth, good food utilization, excellent carcass conformation, wide and long breast muscles and short leg muscles *(Perić et al 2010; Džinić et al., 2011)*.

The extensive use of antibiotics in animal production has increased the risk of development of resistance in human and animal pathogens *(Witte, 1998)*. Because of concerns about potential negative human health consequences, as well as satisfying consumer demand for a food chain free of drugs, use of antibiotics as growth promoters is forbidden in the European Community *(Council Regulation, 1998)*.

The ban on antibiotic usage in Europe lead to increasing researchers interest in finding alternatives to antibiotics for poultry production such as enzymes, organic or inorganic acids, herbs, essential oils, immunostimulators, microelements, probiotics and prebiotics *(Lević et al, 2007)*. Phytogenic additives are a group of natural growth promoters, derived from herbs, spices or other plants *(Hasmeni et al., 2010; Ristić et al., 2008)*. In recent years, the use of phytogenic compounds are increased because their potential role as natural alternatives to antibiotic as growth promoters in animal nutrition *(Mountzouris et al., 2011)*. Phytogenic additives enhance broiler performance and health, and have beneficial effects on: feed intake, broiler growth performances, digestive function, feed conversion ratio, gut health parameters, body weight gain. Also, they may have a beneficial effect on carcass and stored meat quality and production economy *(Cross et al., 2007)*.

Thus, the aim of this study was to determine the influence of phytogenic additives on the economic aspects of broiler production

Material and Methods

The experiment was carried on 16480 broilers, provenience ROSS. Broilers were divided in two groups, control group (C) and experimental groups (E1) and fed under the same conditions for 40 days. Broilers from control group fed with commercial mixtures, while in broilers diet from experimental group E phytogenic additive Biomin P.E.P were added. During whole fattening period, water and feed were provided *ad libitum*.

Calculation of the expense for feed mixtures has been derived according to the standard of expenses for the preparation of animal food, based on market prices

of certain kinds of food and experience normative. The expense for other material has been calculated according to the expenditure made on the observed farm and market prices. Investments into buildings and the equipment have been calculated based on standard investments in objects and equipment. Expenses of the buildings and equipment depreciation have been derived based on the assumed lifetime of the utilized means *(Marko et al., 1998)*. Expenses for salaries were calculated in accordance to realized expenses. Expenses for the energy consumption were calculated on the basis of realized expenditure of the electrical power and fuel. Apart from that, the calculation includes expenses of veterinary and selection services. Calculation of the income was based on clarification of total income from the above mentioned production, whereby the financial result presents the income from the overall production *(Andrić, 1998)*.

Results and Discussion

During the analysis of the observed production, production results have been followed closely. The main production indicators are given in the Table 1.

Table 1. Basic production results for two groups of broilers

Category	C			E		
		price	total		price	total
Chickens	8 240	0.40	3 310.71	8 240	0.40	3 310.71
Produced broilers	**7 796**			**7850**		
Produced broilers, kg	**18 788.40**	1.43	**26 840.57**	**19 232.50**	1.43	**27 475.00**
kg/broiler	2.410			2 471		
Total feed consumption	**33 560**		**16 348.06**	**33 460**		**16 505.81**
Starter	5 100	0.40	2050,02	5 100	0.41	2 082.80
Grover	19 900	0.40	7894,26	19 300	0.40	7 780.31
Finisher	8 560	0.36	3093,06	9 060	0.37	3 331.98
Feed conversion ratio, kg/kg	1.770			1.725		
Average daily gain, kg/day	0.060			0.062		

If we take a look at the Table 1 one can draw a conclusion about the almost minor expenditure of food in group fed with diet with the addition of phytogenic

additives. Also, the same group has bigger number of broilers and average carcass mass at the end of the fattening and achieves higher value of the production.

Table 2. Calculation of the total expenses and the price and benefit calculation, €

Expense category	C	E
Feed expense	16 348.06	16 505.81
Amortization	3 708.00	3 708.00
Salaries	2 158.10	2 158.10
Expenses for energy	1 925.12	1 925.12
Expenses of other and additional materials	824.00	824.00
Total	**24 963.28**	**25 121.03**
Total income	24 963.28	25 121.03
Total expenses	26 804.57	27 475.00
Benefit	**1 870.30**	**2 353.97**
Benefit per broiler	0.23	0.29
Benefit per kg	0.10	0.12
Economy	1.08	1.09
Profitability, %	2.61	3.27

The analysis of the overall economic indicators of the observed production starts from the assessed investment into the farm, in other words investment into the buildings for breading with the following equipment. According to the assessment, the investment into the buildings and equipment amount is up to 370,800.00 €. Calculation of other expenses (energy, work and additional materials) of the production, has been derived per production cycle**.** In the distribution, they were divided proportionally to the starting number of chickens, e.g. two equal groups. In accordance to the derived calculations, establishing of the total expense and the price of the fattening chicken has been derived. Calculation of these indicators is presented in the Table 2.

Calculation of the income includes the incomes that farm achieves and it is based on the sale of broilers. On sale, the price that was achieved was 1.43 €/kg. In the accordance to the number of fattening broilers, average weight reached and the sale price, the calculation of the total income was made.

Based on that the benefit was calculated as the difference between the income and expense.

The profit achieved per one production cycle amounts up to 2,353.97 € for the group fed with the diet with addition of phytogenic additives and 1,870.30 € for the group fed by standard forage mixtures, e.g. 0.29 € and 0.23 € per produced broiler.

If the realized benefit is calculated per kilogram of produced broilers, we get 0.12 €/kg for the group fed with the diet with addition of phytogenic additives, and 0.10 €/kg for the group fed with standard diet. The economy calculated from the ratio of total income and total expenses is 1.09 for the group fed with the mixture with phytogenic additives and 1.08 for the group fed by the standard feeding system.

Profitability of the production is obtained from the ratio of realized benefit and total investment. Total investment includes investments into the buildings and equipment and investment into the unfinished production within the fattening. Thereat, in total five or six production cycles are foreseen per year. Binding of means in the form of debits has not been calculated; instead the calculation has been derived with an assumption of advance payment. Profitability of the overall production process in the observed case is not difficult to calculate, since the production is concentrated and mono-phase. Realized profitability for the group fed with the mixture with addition of phytogenic additives was 3.27% and 2.61% for the group fed with standard forage mixtures.

Conclusion

Economic efficiency and profitability of the production are the most important principles and the basis of rational business in the market economy, which is all and more becoming an imperative for our production too. Economic results of the production of broilers have in the paper been analysed and what can be concluded is the following:

- The profit achieved per one cycle amounts to 2,353.97 € for the group fed with the diet with addition of phytogenic additives and 1,870.30 € for the group fed by standard forage mixtures, e.g. 0.29 € and 0.23 € per produced broiler.
- The economy calculated from the ratio of total income and total expenses is 1.09 for the group fed with the mixture with phytogenic additives and 1.08 for the group fed by the standard feeding system.
- Detailed analysis of economic indicators shows good profitability of the production. The realised profitability of the production makes only 3.27% for the group fed with the mixture with addition of phytogenic additives and 2.61% for the group fed by standard forage mixtures.

Regardless of that, all presented indicators point out the justifiability of the usage of phytogenic additives in the preparation of feed.

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Uticaj dodatka fitogenih aditiva u hranu na ekonomske rezultate tova brojlera

Đ. Okanović, R. Čolović, D. Milić, N. Vukelić, V. Zekić, T. Tasić T., Ikonić P.

Rezime

Proizvodnja brojlera praktično predstavlja najintenzivniju granu stočarstva. Proces reprodukcije relativno je kratak, što omogućava brži obrt angažovanih sredstava. Cilj istraživanja bio je da se utvrdi uticaj smeše sa dodatkom fitogenih aditiva na rezultate tova i ekonomiku proizvodnje brojlera.

Eksperiment je izveden sa pilićima hibrida Ross (N = 16480), podeljenih u dve grupe. Prva grupa (kontrola, C) hranjena je komercijalnom mešavinom hrane brojlera, dok su u eksperimentalnoj grupi (E1) u hranu dodati fitogenih aditivi. Tov brojlera je trajao 40 dana. U podnom sistemu tova hrana i voda su bili stalno dostupni.

Istraživanje ekonomskiih rezultata proizvodnje brojlera se zasniva na odredjivanju ukupnih proizvodnih troškova, vrednosti realizovane proizvodnje i finansiskih rezultata. Obračun ovih ekonomskih kategorija se zasniva na podacima dobijenih sa izabrane farme.

Dobijeni ekonomski rezultati pokazuju merljiva poboljšanja pri korištenju fitogenih aditiva u hrani za životinje u odnosu standardan sistem ishrane: dobit (2 353,97 : 1 870,30 €), dobit po proizvedenom brojleru (0,29 : 0,23 €), ekonomičnost (1,09 : 1,08) i rentabilnost (3,27 : 2,61%).

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CONCENTRATION OF ARSENIC IN WATER AND TISSUES OF BROILERS

M. Živkov-Baloš[1], Ž. Mihaljev[1], S. Jakšić[1], Ž. Ćupić[2], M. Kapetanov[1], S. Obradović[3]

[1]Scientific Veterinary Institute „Novi Sad“, Rumenački put 20, 21000 Novi Sad, Republic of Serbia
[2]Research Institute for Reproduction, Artificial Insemination and Embryotransfer "Temerin", Industrijska zona bb, Temerin, Republic of Serbia
[3] Faculty of Economics and Engineering Management, Novi Sad, Republic of Serbia
Corresponding author: milica@niv.ns.ac.rs
Original scientific paper

Abstract: Water is the primary route of human and animal exposure to chemicals leaching from stores and hazardous waste. Arsenic (As) is a natural non-metallic element found in low concentrations in every part of the environment, including water and food. We investigated the effects of naturally contaminated drinking water with As-levels above the maximum values permitted by the relevant *Regulation* (0.033±0.024 mg/l) on deposition of arsenic residues in organs and meat of broilers. Concentrations of total arsenic in water, feed, organs and meat samples were determined by ICP-MS. Arsenic levels in tissues and organs were within the following range (ppm): liver – 0.009±0.004; testicles – 0.012±0.004; drumstick meat 0.010±0.004; breast meat 0.009±0.004. Chicken meat is an important part of the diet and its consumption is continuously increasing. Thus, safety of animal feed, drinking water and poultry farms conditions are important parameters in assessing the safety of chicken products, which require particular attention.

Key words: total arsenic, water, broilers

Introduction

Arsenic (As) is a natural non-metallic element found in low concentrations in every part of the environment, including water and food. Application of pesticides containing arsenic and other chemical compounds in agricultural practice results in accumulation of this element in the soil and plants and hence the presence of trace amounts of arsenic in food and animal feed (*Shah et al., 2009*).

Inorganic arsenic is generally more toxic than its organic forms. Inorganic arsenic is found in water, which is the potential route of exposure of humans and

animals *(Shah et al., 2009)*. Though it is not always the case, positive correlation between toxicity of arsenic compounds and their solubility in water has been established.

Genotoxicity tests revealed that As inhibits DNA repair and induces chromosomal aberrations *(Schoen et al., 2004)*. After ingestion, 60-90% of As is rapidly absorbed in the gastrointestinal tract. Major part of inorganic As (> 90%) is cleared from the blood very quickly, some part is converted in testes, kidney and lung tissue and some part undergoes metabolic processes in the liver *(Cohen et al., 2006)*. Symptoms of chronic prolonged exposure to low levels of As (arsenicosis) include skin discoloration, chronic indigestion and abdominal pain. Long-term effects include cancers of the skin, lungs, kidneys and liver as well as gangrene-like sores. However, symptoms of chronic exposure to As significantly vary between the individuals, populations and geographic regions suggesting that there is no universal definition of symptoms induced by chronic arsenic poisoning *(Sharpe, 2003)*. Furthermore, some individuals (humans, laboratory animals) can tolerate high arsenic doses, which on the other hand could be fatal for others *(Jones, 2007)*.

Nowadays, the safety of drinking water is one of the most important ecological issues. Water is primary route of transmission and exposure of humans and animals to chemical contaminants originating from facilities for disposal of hazardous waste *(Yang et al., 1989)*. Hazardous waste disposal units, urban and industrial landfills, washing out minerals from natural sources, accidents and agricultural activities are major sources of pollution of surface and ground waters *(Yang and Rauckman, 1987)*. Food and drinking water together account for 99% of the total human intake of arsenic *(Jones, 2007)*. Since present in soil, water, air, plants and all living organisms, the occurrence of arsenic in the food is not surprising. Many kinds of food contain very low amounts of arsenic, whilst highest concentrations were detected in seafood, which is associated with a plankton-based diet of marine species.

Arsenic, along with other naturally occurring minerals, is continuously cycling in the environment, and humans and animals are always and unavoidably exposed to its toxic effects *(Roy and Saha, 2002)*. As is present in all living organisms from *Escherichia coli* to man, thus every organism have developed its detoxification pathways *(Jones, 2007)*.

The objective of this research was to investigate effects of naturally arsenic-contaminated drinking water on deposition of arsenic in different organs and meat of broilers.

Material and Methods

Experimental animals. The experiment lasted for 42 days encompassed a total of 50 Arbor Acres broilers of both sexes originating from the same parent flock. Prophylactic measures, housing, management, diet and water supply during the experimental period were adapted to the floor farming system. During the experiment, the zoo-hygienic and microclimatic conditions were in line with the technological normative, prescribed for this provenience *(Arbor Acres Farm, 1997)*. The feed and water was provided *ad libitum*. Prophylactic measures were applied, and all investigated broilers were subjected to veterinary control that included permanent monitoring and recording of health status changes. At the end of the experimental period (day 42th), seven broilers (both sexes, with equal sex ratio) were sacrificed for organ and tissue sampling.

Experiment. The experimental birds were given free access to water and feed (*ad libitum*). During the experimental period, broilers were fed complete standard mixtures designed according to the age – starter, grover and finisher in the final stage of fattening. Drinking water, naturally contaminated with arsenic, was provided from an Artesian well. Feed and water samples for chemical analysis were collected immediately before the beginning of the experiment.

Methods of chemical analysis. Total arsenic concentration in samples of water, complete premixes, organs and meat of broilers was determined by the ICP-MS method. The samples were prepared by the use of microwave digestion method. Arsenic (He-M, IT 1 s/P) was determined on Agilent ICP-MS 7700 by applying the coupled plasma technique through isotope ^{75}As *(Dolan and Capar, 2002; Wu et al., 1997)*.

Statistical analysis. The obtained results were grouped into corresponding statistical series and analyzed according to standard ANOVA procedure using the software package SPSS 8.0 for Windows.

Results and Discussion

Results of the analysis of drinking water and feed mixtures for experimental broilers were displayed in Table 1. Average content of As in the examined drinking water samples (0.033±0.024 mg/l) was above the maximum permissible level (0.01 mg/l) set by the *Regulation (1998; 1999)*.

Table 1. Total arsenic content in drinking water and complete premixes for diet of experimental broilers

Examined sample	n	As content (mg/kg)		
		Mean	Stand. deviation	Variation interval
Water	5	0.033	0.024	0.0126 - 0.3205
Complete mixture I	3	0.003	0.001	0.001-0.003
Complete mixture II	3	0.005	0.001	0.001-0.005
Complete mixture III	3	0.003	0.001	< 0.001-0.003

As content in complete mixture samples ranged from < 0.001 up to 0.005 mg/kg, which was significantly lower in the comparasion to the maximum permissible level (2 mg/kg) set by the *Regulation (2010)*. Though presence of this toxic element was evident at detectable and measurable levels, its concentrations were significantly lower in the comparasion to some previous research on animal feed *(Živkov-Baloš et al., 2007, 2011)*. Thus, As intake through food was minimized, that was in the line with nutritional recommendations. Research performed on various animal species including chicks has demonstrated essential importance of arsenic. Nutritional recommendations for arsenic supplementation are 0.025-0.050 mg/kg of animal feed *(DeSesso et al., 1998)*.

Impact of contaminated water on poultry production is extremely difficult to estimate. Data available in the literature offer only limited information about effects of water contaminants on reproductive performance and egg quality *(Vodela et.al., 1997)*.

Experimental broilers revealed harmonic body composition, properly developed bone and muscle tissue, were lively and in good condition. Feathers, skin and visible mucosa were normal, without any distinctive marks. The appetite was good, and faeces were normally formed. Ability of active moving and coordination were harmonized, muscle tonus pronounced and preserved. Mortality rates at particular experimental stages were within the range of technological normative for Arbor Acres provenience *(Arbor Acres Farm, 1997)*. Results on total arsenic content in organs and meat of experimental broilers are presented in Table 2.

Table 2. Total arsenic content in organs and meat of experimental broilers

Examined sample	n	As content (mg/kg)		
		Mean	Stand. deviation	Variation interval
Drumstick meat	6	0.010	0.004	0.007-0.017
Breast meat	6	0.011	0.004	0.006-0.015
Liver	6	0.009	0.004	0.001-0.014
Testicles	5	0.012	0.004	0.008-0.016

Arsenic content in broiler meat samples (drumstick, breast) was within the ranges of 0.007-0.017mg/kg and 0.006-0.015, respectively, which is significantly lower than the maximum permissible level (0.1 mg/kg) set by the *Regulation (2011)*. In liver and testes samples, the total As content ranged from 0.001 to 0.014 mg/kg and 0.008 to 0.016 mg/kg, respectively. This is also significantly below the maximum permissible value of 0.5 mg/kg. Various quantities of total As content detected in all examined tissue samples, showed quite consistent concentration irrespective of the tissue type. Since widely distributed in the environment – soil, animal feed and water - the detection of arsenic in birds' tissues is highly probable *(Desheng and Niya, 2006)*. According to the *National Academy of Sciences (1977)*, the content of As in chicken meat, as the most common foodstuff, is 0.02 ppm of dry matter *(Jones, 2007)*. A study conducted at the U.S.A. market in the period 1991-1996, the content of total As in muscle tissue of broilers was 0.030-0.086 µg/g *(Tao and Bolger, 1999)*, whereas *Dabeka et.al. (1993)* reported the content of total As in chicken meat from Canadian market, being 0.029 µg/g. Data of As content in broiler meat obtained in our research are in accordance with results of other authors. Experiment performed on broilers consuming water contaminated with the mixture of chemical components (arsenic, cadmium, lead, benzene and trichloroethylene) at low (0.80, 1.3, 5.0, 6.7 and 0.65 ppm) and high concentrations (8.6, 13, 50, 67 and 6.5 ppm) revealed that As contents in bones, liver and kidneys were below the limit of detection for the applied method, i.e. below 0.1 ppm *(Vodela et al., 1997)*. The results of our research fairly correspond with the data reported by *Vodela et al.(1997)*; however, concentrations of As in drinking water were significantly different. Supplementation of arsenic in the diet appears to be more significant factor when the level of toxic metals in drinking water is lower. It was observed that laboratory experiments on animals exposed to low doses of arsenic in drinking water frequently give false results. This is mainly due to the overlooking of arsenic in animal feed, which is present as a contaminant and not as a feed additive *(Mitchell et al., 2011)*. This thesis was confirmed in an experiment on Japanese quails reported by *Desheng and Niya (2006)*. Their experiment with Japanese quails that were fed diet supplemented with 50 mg and 100 mg/kg 4-arsanilic acid and drinking water containing 0.008 mg/l of As revealed an uneven distribution of arsenic in the tissues. Namely, As content in muscle tissue (drumstick meat, breast) was relatively low, not exceeding the limit of 0.64 mg/kg of dry matter. Arsenic content in liver, heart and kidneys was significantly higher ranging from 0.81 to 2.82 mg/kg of dry matter, whereas highest levels were recorded in the liver. As-concentration in tissues and organs increased with the level of arsanilic acid supplemented in the feed. The authors reported that As was detected in tissues of quails from the control group, which was fed feed containing basal-natural level of arsenic and water containing 0.008 mg/kg of As. In that

respect, they concluded that As has accumulated in the tissues of birds disregarding its concentration in feed and drinking water.

Conclusion

Chicken meat is the most popular meat in human diet worldwide. In that respect, arsenic ingested in chicken meat and entrails makes an important portion of the total arsenic intake in humans. Our investigation revealed that using drinking water with unallowable arsenic content did not affect the safety of chicken meat for human consumption with respect to As concentration in the meat. Of course, the period of animals' exposure to elevated levels of arsenic still remains an important issue in a view of safety of meat and entrails of older birds. Some further research of this issue, as well as dietary interactions of toxic elements in the food and drinking water and their simultaneous effects on animal health, reproduction and safety of products for human consumption.

Meat and entrails of poultry are an important element of human diet, showing increasing tendency worldwide. Thus, the safety of animal feed and drinking water and technological conditions on poultry farms are important parameters when assessing the safety of poultry products.

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Koncentracije arsena u vodi i različitim tkivima tovnih pilića

M. Živkov-Baloš, Ž. Mihaljev, S. Jakšić, Ž. Ćupić, M. Kapetanov, S. Obradović

Rezime

Voda je primarni put prenosa i izloženosti ljudi i životinja hemijskim kontaminentima koji potiču sa skladišta opasnog otpada. Arsen (As) je nemetal koji se u niskim koncentracijama može naći u svakom delu životne sredine, uključujući vodu i hranu. Ispitivan je uticaj prirodno kontaminirane vode za piće u kojoj je koncentracija ukupnog As bila iznad Pravilnikom makimalno dozvoljene vrednosti (0,033±0,024 mg/l), na deponovanje arsena u različitim organima i mesu tovnih pilića. Koncentarcije ukupnog arsena u uzorcima vode, hrane, organa i mesa pilića

određena je metodom ICP-MS. Koncentracije arsena u tkivima i organima kretale su se usledećem rasponu (ppm): jetra: 0,009±0,0,004; testisi: 0,012±0,004; meso batka sa karabatkom 0,010±0,004; belo meso 0,009±0,004. Pileće meso je važan deo ishrane i njegova potrošnja je u stalnom porastu, zato je važno, pri procenjivanju njegove bezbednosti, uzeti u obzir - bezbednost hrane za životinje, vode za napajanje, kao i tehnološke uslove živinarske farme.

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DETECTION OF FLUOROQUINOLONE RESIDUES BY MICROBIOLOGICAL SCREENING METHOD – FLUMEQUINE

J. Petrović[1], V. Đorđević[2] , I. Stojanov[1], D. Milanov[1], S. Stefanović[2]

[1]Scientific Veterinary Institute „Novi Sad“, Rumenački put 20, 21000 Novi Sad, Serbia
[2]Institute of Meat Hygiene and Technology, Kaćanskog 13, 11 000 Belgrade, Serbia
Corresponding author: jelenap@niv.ns.ac.rs
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Abstract: Systematic control of antibiotic residues includes analysis and selection of large number of samples, and requires a wide range of screening methods. Screening tests should satisfy the following requirements: they must be capable to detect antibiotics of interest, detection limits must comply with the requirements (MRL- maximum residue limit), they must be easy to perform and cost effective, test results are to be obtained rapidly, and the tests must be standardized. The aim of this study was to examine the performance of screening test microbiological method with *E. coli* as test microorganism: capability to detect fluoroquinolone – flumequine at MRL levels in both fortified and incurred chicken and fish tissue samples. LOD (limit of detection) of microbiological method were determined in tissue samples fortified with flumequine. Incurred samples were obtained in experimental design where chickens were treated with therapeutical doses of flumequine. The presence of fluoroquinolones in muscle and liver was detected by microbiological and HPLC method. Examination results revealed detection limits of the microbiological method ranging from 300 ng/g to 400 ng/g for flumequine. Examination of treated animals using microbiological screening method gave positive results in all samples where the residues content was above MRL level. The results of examination of the flumequine residues in tissues of treated animals using screening microbiological method entirely fulfill the demands of a qualitative method.

Key words: fluoroquinolones, flumequine, residue, screening

Introduction

The fluoroquinolone antimicrobials belongs to a class of *semi*-synthetic agents that are important in both human and veterinary medicine. Flumequine is fluoroquinolone antimicrobial developed exclusively for the use in veterinary

medicine. Common poultry infections, such as mycoplasmal infections, colibacilosis and pasteurelosis, frequently are treated with this drug as well as fish infections caused by *Aeromonas salmonicida, Vibrio anguillarum, Yersinia ruckeri, Renibacterium salmoninarum, Pasterurella piscida (della Roca et al., 2004; Martinez et al., 2006)*.

The widespread use of fluoroquinolone compounds as therapeutic and prophylactic agents, particularly in intensive poultry production, has become a matter of great concern in recent years due to the identification of resistant *Campylobacter* and *Salmonella* strains in meat and possible transfer to humans via food chain *(Petrovic et al., 2008)*. MRL values for flumequine in EU are 400ng/g and 800ng/g for chicken meat and liver, 600ng/g for fish meat *(Council Regulation 2377/90)*.

A number of methods have been developed for the analysis of fluoroquinolones in animal tissues, some of them rely on HPLC with ultraviolet and fluorescence detection *(Marschiello et all., 2001)*, automated microdialysis-liquid chromatography *(Schneider, 2001)* and LC-MS *(Schneider and Donoghue, 2002)*. Systematic control of antibiotic residues in European Union includes analysis and selection of large number of samples, and requires a wide range of screening methods. Samples that are positive or suspect as to the presence of residues are further analyzed using confirmatory methods (HPLC, LC/MS). Introduction of sistematic control of residues is one of important steps which will help our broiler and fish production to reach European standards. According to *Petrovic et al. (2012)* it is necessary to build efficient livestock production that can compete in the European market contributing to the growth of farmers and national income.

Screening tests must satisfy the following requirements: they must detect antibiotics of interest, detection limits must comply with the requirements (MRLs), they must be easy to perform and cost effective, test results are to be obtained rapidly, and the tests must be standardized (low variability within and between batches/laboratories) *(Suhren and Heeschen, 1996)*. Microbiological inhibition tests are widely used as a standard for screening purposes. The test principle is based on measurement of the inhibition zone, which presents the inhibition of multiplication of test microorganism in presence of antibiotics. These tests can serve as rapid tests as the result can be obtained within 24 hours *(Petrovic et al., 2008)*.

The aim of this study was to examine the performance of screening test microbiological method with *E. coli* as test microorganism: capability to detect fluoroquinonolens at MRL levels in both fortified and incurred chicken and fish tissue samples. LOD of microbiological method was determined in tissue samples fortified with flumequine. Incurred samples were obtained in experimental design

where chickens were treated with therapeutical doses of flumequine. The presence of fluoroquinolones in breast muscle and liver was detected by microbiological and HPLC method.

Material and Methods

Chemicals and reagents. Flumequine analytical standards was purchased from Sigma Company, USA. In our experiment we used preparation Flumekvin® pulv ad us.vet. (Hemovet - Serbia), 100 g of powder contains 10 g of flumequine.

Microbiological method: Test agar pH 8.0 was prepared in our laboratory (Caseine hydrolisate 2%, dextrose 0.4%, NaCl 1%, agar agar 1.6%). *Escherichia coli* NCIMB 11595 was used as test microorganism. Paper disks containing 0.003 ciprofloxacin µg/disk (Mast Diagnostic, Mereyseyside, UK) were used as positive control.

HPLC/Fl: Methanol, acetonitrile, n-hexane and phosphoric acid were purchased from J. T. Baker, Holland. All the solvents were of HPLC purity. Waters "Sunfire" column, C18, 150x4.6mm, 3.5µm particle size was used for separation at flow rate for flumequine of 0.7 mL/min. Mobile phase (0.01M phosphoric acid (pH 3)/acetonitrile; 80:20 v/v1-10. min and 60:40 - 10-20 min) was used for the elution.

Determination of LOD – fortified samples. The limit of detection (LOD) of the microbiological method was determined by the method recommended by *Reichmuth et al.(1997)*. Series of 7 concentrations of antibiotic were analyzed in 12 replicates. Meat without antibiotics and meat fortified with 2-3 times higher concentration of antibiotics then expected limit of detection were used as negative and positive controls, respectively. Expected LOD was determined in preliminary examinations. Three different concentrations between the negative control sample and expected positive sample were analyzed. The following concentrations were examined (ng/g): flumequine 0.00, 3.12, 6.25, 12.50, 25.00, 50.00, 100.00, 200.00, 400.00, 800.00 and 1600.00. The results are shown in the form of dose-response curve. For this examination LOD is defined as that concentration, where 95% of the results were evaluated positive. LOD was determined by plotting the line for 95% positive responses. The place where the line cuts the dose-response curve presents LOD. Fish samples were fortified with ¼ MRL, ½ MRL, 1MRL, 2 MRL, 4MRL, MRL is for fish 600ng/g.

Animals, drug and protocol of study – incurred samples. The study was performed on 65 healthy chickens (Arbor acres); 1-day old chickens were included in the experiment, at the age of two weeks the chickens were randomly divided into two groups. Group A (30 animals) was the control group, which was not treated with antimicrobials and group B (35 animals) was treated group. At the age of 28 days the chickens in group B were started with therapy (12 mg/kg bw/day). Drug

were given via drinking water, for five consecutive days. The chickens were euthanized during the withdrawal period, at each sampling three chickens were euthanized, the samples of breast muscle and liver were obtained. The samples were stored at -20^0C until assayed for the presence and concentrations of flumequine.

Qualitative analysis: microbiological method. Test agar pH 8.0 was seeded with *Escherichia coli* NCIMB 11595. Working solution of *E. coli* NCIMB 11595 was made of freshly prepared culture. The culture was diluted in peptone-salt solution to give optical density of 0.452 at 620 nm in a 10 mm cell, with the use of peptone-salt solution as a reference. Sterile Petri dishes were filled with inoculated test agar. All plates were subjected to a quality control: paper discs containing 0.003 ciprofloxacin µg/disk were placed in the center of the Petri dish. Meat and liver were sampled while still frozen, an 8 mm diameter cork borer was used to remove a cylinder of frozen meat. The meat cylinders were cut into 2 mm thick discs. Four discs of meat/liver were placed on opposite ends of the plate. Each sample was examined in 12 replicates. The plates were kept in refrigerator for 2 hours and than incubated on 37^0C for 24 h. After incubation the plates were inspected for inhibition zones around the meat/liver discs and inhibition zones (IZ) were recorded (2 mm width was considered positive result).

Quantitative analysis – HPLC with fluorescence detection. HPLC method with fluorescence detection at excitation wavelength of 312 nm and emission wavelength of 366 nm *(Ramos et al., 2003)* was used for determination of flumequine residues in meat and liver. The detection for flumequine limit is 20ng/g and quantification limit is 50ng/g. Flumequine was detected by gradient elution in 20 minutes. Quantification was performed using external standard method and the results were obtained from the calibration curve of blanks fortified at four levels of flumequine.

Statistical analysis. Statistical analysis was performed using the Microsoft Office Excel 2000 and statistical software SPSS for Windows 8.0.0. Screening method data were analyzed by the use of descriptive statistic methods. Differences in IZ diameters were analyzed for statistical significance by the use of Student's t – test. The differences of $p<0.05$ were considered significant.

Results and Discussion

Figure 1 demonstrates the results of the examination of the microbiological method sensitivity in chicken muscle towards flumequine in the form of dose-response curve. Concentrations 0.00 - 100.00 ng/g of flumequine did not have any positive response, while the concetrations 200 and above gave 50-100% positive responses. For this examination, LOD was defined as concentrations, where 95%

of the results were evaluated as positive. LOD of flumequine can be derived from figure 1as 400 nf/g for flumequine. For fish muscle samples similar LOD was found because concentration of 300 ng/g had 100% of positive responses in Table 1.

Chicken breast muscle and liver samples from chickens from experiment sampled during widhdrawal period were analyzed by the microbiological and HPLC methods for flumequine presence and concentrations, and the results are shown in Table 2 and Figure 2.

Table1. Fish muscle fortified fish with flumequine

Flumequine MRL 600ng/g	Microbiological method (IZ in mm)				
	x	SD	SE	Cv	Iv
4 MRL	8.29	0.26	0.07	3.11	0.5
2 MRL	8.25	0.26	0.08	3.17	0.5
1 MRL	7.38	0.31	0.09	4.21	1.0
1/2 MRL	3.67	0.33	0.09	8.88	1.0
1/4 MRL	0	0	0	0	0

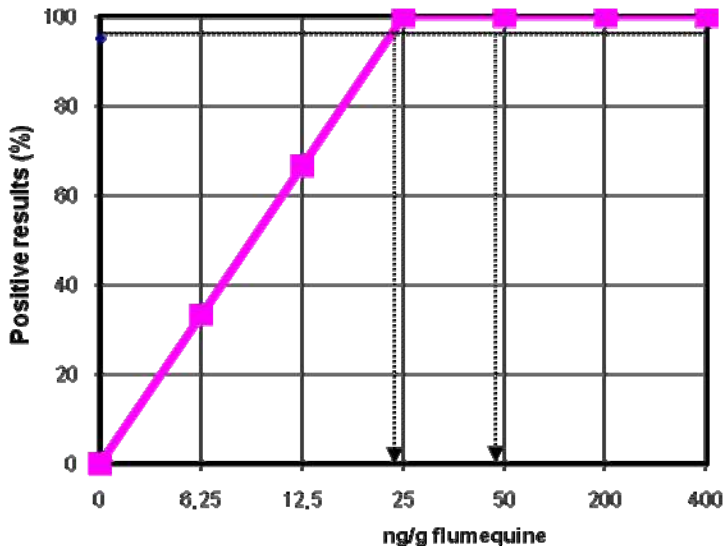

Figure 1. LOD of microbiological method for flumequine

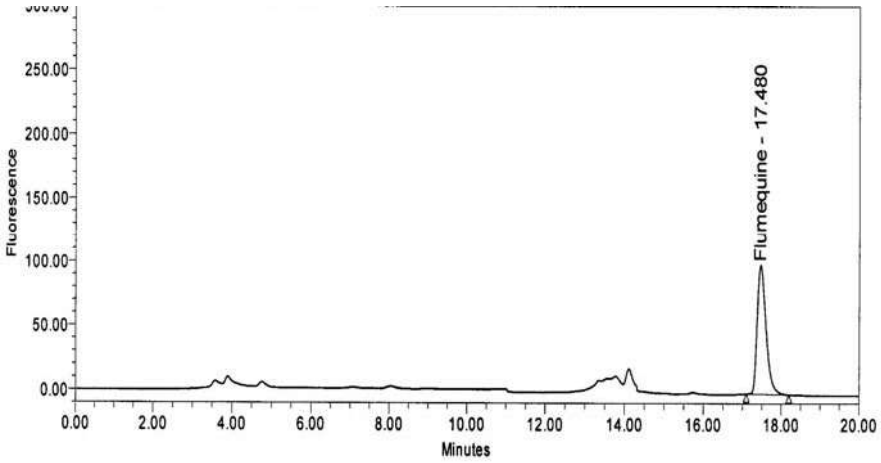

Figure 2. Chromatographic determination of flumequine residues in chicken muscle

Table 2. Determination of residues during flumequine administration

Treatment day		Microbiological method (IZ in mm)							HPLC (ng/g)
		x	SD	SE	Cv	Iv	t	% posit	Flumequine
1 PT	M	3.71	1.429	0.292	38.53	2.00	3.59*	100	980
	L	5.46	1.911	0.390	35.00	7.00		100	1760
2 PT	M	0.00	-	-	-	-	-	0	60
	L	0.00	-	-	-	-		0	100

M- meat; L- liver; -PT- day after the end of therapy; * – significant difference ($p < 0.05$),

Limit of detection is the basic parameter in determining the test sensitivity. Test sensitivity is the probability of obtaining positive test result in truly positive samples. In a view of antimicrobial residue detection in food, a positive sample is the sample that contains residues at level above the MRL. This value is the basic parameter for sample assessment, since samples containing residues below MRL level are considered negative, i.e. safe. An ideal screening test would yield a LOD exactly at MRL level for each particular antimicrobial. However, performing of such tests is not always feasible in daily practice. Thus, the test is considered enough sensitive if the detection limit is at or below the MRL level, an never above the MRL. The LOD of a microbiological test depends of the innate sensitivity of the test bacterium, pH and thickness of growth medium *(Petrovic 2006)*.

The results obtained in this research indicated detection limits of microbiological inhibition test towards flumequine being between 300 and 400 ng/g, respectively in fish and chicken muscle. According to *Okerman et al. (1998 a,b)* detection limits of the pH6 plate *E. coli* ATCC 11303 were 1000 ng/g towards flumequine. In 2001, the same authors investigated sensitivity of another strain of

E. coli- Bayer 14 and established detection limits of 150 ng/g. Similar data were reported by other researchers. *Kibis and Marinsek (2004)* applied microbiological inhibition method with *E. coli* ATCC 25922 as a test microorganism. Examination of poultry meat containing 400 ng/g flumequine revealed positive result in 80% samples, whereas concentration of 450 ng/g resulted in 93.3% positive responses. Sensitivity differences that occur in various authors are mainly related to diverse strains of *E. coli* as well as to differences with respect to test-design (nutritive medium, incubation temperature).

Examination of negative control samples did not revealed any false positive response. The established detection limit corresponds with MRL-values toward flumequine in poultry meat and liver and fish meat. Samples with residue concentration within of above MRL examined by microbiological inhibition method revealed 100% positive results.

After oral application, fluoroquinolones are well absorbed, distributed into tissues and excreted in urine and feces at high concentrations *(Prescott et al., 2000)*. Flumequine is excreted in the urine and faeces as the parent drug (80%), glucuronide conjugates (12.5%) and 7-hydroxyflumequine (6%), *(EMEA, 1996)*.

During the post treatment period, flumequine concentrations in breast muscle and liver exceeded the MRL values until 2-d of withdrawal period. Rapid decline of flumequine residues in broiler edible tissues was found in our experiment. Similar data were presented in the *EMEA report (1996)*, chickens were treated with equal flumequine doses like in our experiment (12mg/kg/day for five days), 6 hours after cessation of treatment, the concentrations of flumequine were significant: 1 500 ng/g in muscle, 720 ng/g in skin/fat and 2 450 ng/g in liver. Flumequine was gradually eliminated from the chicken's body, after the treatment was finished *(EMEA, 1996; Prescott et al., 2000)*. After 48 hour withdrawal period, the concentrations of flumequine were below 170ng/g in our experiment, as well as in *EMEA report (1999a,b)*. On the second day no muscle and liver samples had positive response in microbiological assay. Low levels of flumequine residues were detected in these samples, much below MRL as well as much below the level of detection of microbiological method. On the first day after the end of withdrawal period flumequine was detected by HPLC method in meat (40ng/g) and liver (90 ng/g). Similar results are found in EMEA reports (1996), where seventy two hours after the end of the treatment only the traces of flumequine could be detected in all broiler tissues.

Two-day withdrawal period for flumequine allowed time for the residue concentration in meat and liver to decrease to an acceptable level prior to slaughter (below MRL).

Conclusion

The results of examination of the flumequine residues in tissues of treated animals using screening microbiological method entirely fulfill the demands of a qualitative method. Examination of treated animals using screening method gave positive results in all samples where the residues content was above MRL level.

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Detekcija rezidua fluorohinolona primenom mikrobiološke skrining metode – flumekvin

J. Petrović, V. Đorđević , I. Stojanov, D. Milanov, S. Stefanović

Rezime

Sistematska kontrola rezidua antibiotika uključuje analizu i selekciju velikog broja uzoraka i zahteva primenu različitih skrining metoda. Skrining testovi treba da ispune sledeće zahteve: moraju imati sposobnost detekcije antibiotika od interesa, prag detekcije mora biti saglasan sa zakonskim okvirima (MDK- maksimalno dozvoljena koncentracija), moraju biti jednostavni za izvođenje i isplativi, rezultati testa treba da se dobijaju brzo, testovi moraju biti standardizovani. Cilj ovog rada je da ispita performanse mikrobiološke skrining metode sa *E. coli* kao test mikroorganizmom: sposobnosti da detektuje antibiotik od interesa fluorohinolon – flumekvin u MDK količinama u obogaćenim i u uzorcima iz ogleda (tkiva pilića i riba). Prag detekcije (LOD) mikrobiološke metode je određen u uzorcima tkiva obogaćenim sa flumekvinom. Uzorci tkiva iz ogleda su dobijeni eksperimentalnim ispitivanjima na pilićima koji su tretirani propisanim terapijskim dozama flumekvina. Prisustvo fluorohinolona u mesu i jetri je ispitano mikrobiološkom i HPLC metodom. Ispitivanjima je ustanovljen prag detekcije mikrobiološke metode u opsegu od 300 ng/g do 400 ng/g flumekvina. Ispitivanjem lečenih životinja mikrobiološkom metodom su dobijeni pozitivni rezultati za sva tkiva u kojima je sadržaj rezidua bio iznad MDK. Rezultati

ispitivanja rezidua flumekvina u tkivima lečenih životinja primenom mikrobiološke skrining metode u potpunosti ispunjavaju zahteve za kvalitativne skrining metode.

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PREDISPOSING FACTORS OF CLOSTRIDIOSIS IN COMMERCIAL POULTRY FLOCKS

M. Kapetanov, D. Potkonjak, M. Živkov Baloš, I. Stojanov, D. Milanov, Željko Mihaljev

Scientific Veterinary Institute Novi Sad, Rumenački put 20, 21000 Novi Sad, Serbia
Corresponding author: milos@niv.ns.ac.rs
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Abstract: The poultry enteritis complex caused by anaerobic bacteria from the genus *Clostridium* is comprised of two entities: the ulcerous enetritis seu „quail disease“ and necrotic enteritis, infections with *Clostridium perfringens* type C and A, respectively. All poultry species and categories are susceptible to infection with clostridia, that are common residents in the digestive tract. The infection tend to spread horizontally, mostly through feces. Due to the ubiquitous nature of the microorganism, several predisposing factors are necessary to provoke the disease, such as errors in technology, nutrition or management. Abberations in nutrition that cause perverse apetite – litter eating, that lead to incomplete obstructions in the digetive tract, and diets based on grains without adding sand and gravel particles, all can stimulate over multiplication of clostridia and destruction of the intestinal mucosae. Other diseases can also create suitable environment for costridia to multiplicate and express pathogenic effects. Outbreaks of clostridiosis are particularly closely related to subclinical coccidiosis, and certain concomitant infections with viral agents, including Infectios bursal disease virus and Chicken anemia virus. The clostridial spores, that are highly resistant in the environment and to numerous disinfectants, enable the spread and long persistence of the infection. In the study, frequency of major predisposing factors in the reported outbreaks of clostridial infections in poultry was analyzed. Cases of clostridiosis in breeder flocks and fattening chickens and turkeys were described in detail, all recorded in the South-Bačka and Srem epizootiological regions.

Key words: clostridiosis, poultry, predisposing factors

Introduction

The poultry enteritis complex caused by anaerobic bacteria from the genus *Clostridium* is comprised of two entities: the ulcerous enetritis seu „quail disease“ and necrotic enteritis, infections with *Clostridium perfringens* type C and A,

respectively. They all produce exogenous and endogenous toxines, with *alfa* toxin and *beta* toxin predominantly in *Cl.* perfringens type A and C, respectively *(Nauerby et al., 2003; Gholamiandekhordi et al., 2006).*

All poultry species and categories are susceptible to infection with clostridia. Ulcerous enteritis is common in commercially raised chicken, quail, pheasant and partridge. Waterfowl is not susceptible to „quail disease". It is seen most frequently in offspring, in chickens and quails at age 2 to 12 weeks, and in turkeys at 3 to 8 weeks of age. The disease often coincide or outbreaks after coccidiosis, infectious anemia or some stressful conditions, and may last approximately 3 weeks, even 6 to 10 months. Mortality rate in chickens is about 2 to 12%, and can be lower (1 to 5%) if treated. The highest mortality was recorded in quails after 5 to 14 days and can reach 100%. Clinical signs include sudden increase of mortality, depression, ruffled feathers and diarrhea. Patches of necrotized mucosa may be present in feces. In turkeys vocalization is changed. Major lesions are found in small intestine (jejunum), such as dylated intestines, deeply damaged and smelly. Localized or general pseudomembranous covering of intestinal mucosa is yellow, so called „turkey towel". On histopathology coagulation necrosis is found.

The infection spreads horizontally in the environment with contaminated feces. Clostridia are common residents in the digestive tract. Frequently they can be found in dust, dirt, insect larvae and endogenous parasites. Thus predisposing factors contribute the disease outbreak: poor hygiene and care, particularly in intensive farming, irregular carcass removal, improper disinfection, poor nutrition and changes in diet *(Kaldhusdal and Skjerve, 1996; Dahiya et al., 2006; Olkowski et al., 2008; Keyburn et al., 2008)*. Litter eating causing partial obstructions in the gut and nutrition based on grains without addition of grit *(Pelagić et al., 2000)* are reported as major predisposing factors. Others include subclinical coccidiosis *(Williams, 2005)*, Gamboro disease and infectious anemia *(Orlić et al., 2003)*. Due to production of spores that show resistance to environmental conditions and most of disinfectants, bacteria can survive in nature prolonged time. In the study, frequency of major predisposing factors to clostridiosis was analyzed. Detail cases of clostridiosis that were recorded in regions of South Backa and Srem, in commercial breeder and fattening flocks.

Material and Methods

In the study, the folowing species and categories were included: broiler chickens and turkey poults raised in big conglomerate, pheasant chickens, breeder flocks of meat- and egg-type during rearing and exploitation. The isolation of clostridia was performed on case-record basis.

Clostridium sp. were isolated from liver tissue. Tissue was added to semisolid Thioglycolate medium and preheated at 70◦C for 10 min and afterwards incubated at 37◦C, for 24 h . Content was subsequently plated on blood agar, parallel in aerobic and anaerobic conditions. Suspect colonies of gram positive bacilli that showed growth and beta hemolysis in anaerobic conditions were further confirmed with biochemical reactions *(Quinn et al., 1998)*.

Results and Discussion

Poultry enteritis caused with Clostridium sp. is complex multifactorial disease, and predisposing factors influence the occurance and severity, including some still unknown. In our investigations, clostridiosis was often diagnosed in absence of growth promoters, regular vaccination against coccidia, enzootic aflatoxicosis, and aberrations in menagement, technology and improper content or quality of fibres in diets. In the following text, most frequently recorded predisposing factors are described.

Antibiotics. Drastic increase in *Cl.* perfringens necrotic enteritis incidence is attributed to ban of antibiotics as groth promoters supplement in diets: bacitracin, virginiamycine and avilamycine. Also incidence of ulcerous necrotic enteritis increased in young breeders of meat- and egg-type and in broilers. The outbreaks were recorded as early as three weeks of age, in acute course and high morbidity and mortality rates of 8 to 12 %, despite treatment. In older poultry symptoms varied. In case of mixed infections, clostridiosis was more pronounced. Economical losses in case of clostridiosis in early age are huge *(Kaldhusdal and Lovland, 2000)*. Alternative solutions are subject of intense research, in order to prevent or treat and control the disease, like fat acids and essential oils *(Giannenas et al., 2003; Leeson et al., 2005; Timbermontl et al., 2009)*.

Coccidial infections. Infections with coccidia are well known predisposing factor to clostridiosis, due to severe lesions in digestive tract. Immunization against coccidia is practically the only alternative to anticoccidial chemotherapy. In recent decades there is increased interest in immunoprophylaxis *(Orlić et al., 1997)* since anticoccidial preparations bring issues like residual content in meat and antimicrobial resistance when used unplanned. Some errors were observed in vaccination practice, for example, residual anticoccidials in diets. Many feed producers are not equipped to provide separate lines to ensure absense of the anticoccidials in diet. Limitted tools to prevent immunosuppressive substances including mycotoxins and some viral infections may provoke coccidiosis. Typical clinical signs and pathological findings were not so rarely seen in rearing flocks, two weeks after vaccination against coccidiosis. Bacteriological investigations marked clostridia to be primary pathogens, instead of clostridia, however,

approximately 20 days later, clostridia overtake the leading role in clinical and pathological changes, as shown in Photographs 1,2 and 3 *(Kapetanov, 2011; McDougald, 2008)*.

Photograph 1. Cross section of doudenum.

Photograph 2. Necrotic enteritis

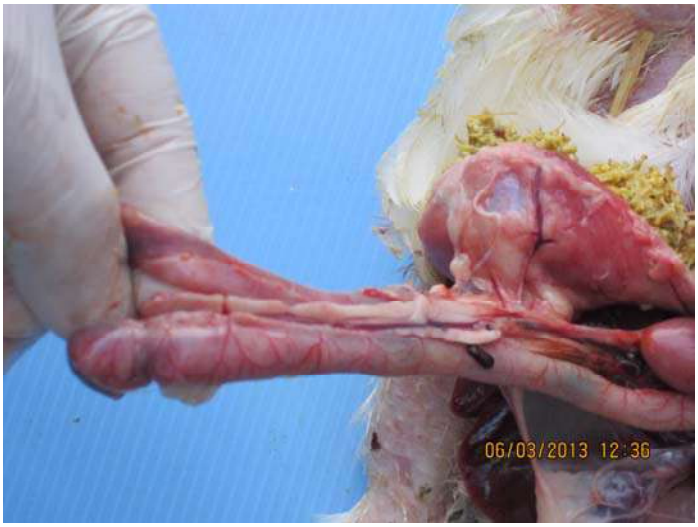
Photograph 3. Necrotic debris on intestinal epithelium

Mycotoxins. As natural contaminents, most frequently occur: aflatoxins, ochratoxins, trychotecenes, fumonizins, zearalenon etc. During period from year 2012 to 2013, there was a real enzootic aflatoxicosis in poultry with severe effects on poultry health. During investigations, some interesting findings were recorded. One of the almost readilly present isolates were of *Cl.* perfringens. On section, unspecific to severe necrotic enteritis in proximal parts on small intestines were recorded in fattening turkeys *(Kapetanov et al., 2013)*.

Technology and menagement on rearing and production breeder farms. In less experienced farmers, abberations in technological guidelines are frequent. Decreased consumption due to insufficient feeding space or inadequate feed distribution may lead to litter eating, particularly during litter refreshment with straw or shavings. In such circumstances, clostridiosis in a form of barn infection was often seen (Photographs 4, 5 and 6).

Photograph 4. Typical *postmortem* position of turkeys.

Photograph 5. Content from digestive tract, mainly straw.



Photograph 6. Inflamation of duodenum serosa.

In period of feeed restriction during rearing, an old habit to add grains (barley or oats) over litter instead in feeders. This enables consumption of grains that are contaminated with clostridia and other potential pathogens. In our investigations, mainly subclinical clostridiosis was recorded during feed restriction period. Increased number of birds with growth retardation and decrease of flock uniformity was found. Additionally, physical and sexual maturation were prolonged as well as increased number of smaller eggs unsuitable to hatch *(Kapetanov et al., 2011a)*.

Content and quality of fibres in complete diets. Raw fibres, sometimes described as non-starch ploysacharides, may be of small nutritional value to poultry, but are favourable to health since enable proper and healthy digestive tract. If used in later rearing phase, positive effects are seen in gut development and appetite. This is very useful in young layers, particularly at the onset of lay. Grains and their by-products (bran) or by-products of oilseeds (sunflower meal, rapeseed meal) are good sources of fibre. Dissolved dried grains also can be used. Other raw materials, rich in fibres, are useful, but only in content that doesn't lower energetic level. Classical feeding regimen based on corn and soy, can hardly satisfy recommended fibre content. In categories that have high protein requirements slower intestine motility occurs if celulose fibres are low. Optimal celulose fibres influence: better digestibility, decrease of undigested feed and decreased feed flow. High amounts of undigested feed in small intestines and slow feed flow, increases possibility for fast spread and colonization of facultative anaerobic bacteria. This is the main reason for need to have 5 to 6% content of raw fibres in diets during

rearing. Many commercial preparations of raw fibres can be found on market *(Williams, 2005; Gholamiandehkordi et al., 2007)*.

Conclusion

Averseness of the predisposing factors that lead to colstridiosis enables to alleviate and control, even eliminate the disease.
Modern industrial poultry production needs alternative solutions to prevent and control slostridiosis. Solutions that are available on market include certain fatty acids and essential oils.

One of the prevention possibilities may rely on production of vaccines with attenuated or inactivated strains and toxoids.

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Predisponirajući faktori pojave klostridijalnih infekcija u jatima živine

M. Kapetanov, D. Potkonjak, M. Živkov-Baloš, I. Stojanov

Rezime

Kompleks enteritisa živine uzrokovane anaerobnim bakterijama iz roda Clostridium sačinjavaju dva entiteta: ulcerozni enteritis ili prepeličija bolest, uzrokovana Clostridium perfringens tip C., i nekrotični enteritis, uzrokovan Clostridium perfringens tip A. Na klostridijalne infekcije osetljive su sve vrste i kategorije živine. Infekcija se širi horizontalnim putem, pri čemu je feces glavni kontaminent okoline. Klostridije su ubikvitarni mikroorganizmi i česti stanovnici digestivnog trakta živine. Zbog toga značajnu ulogu u izbijanja bolesti uglavnom imaju predisponirajući faktori: držanje, ishrana, nega i smeštaj živine. Posebnu predisponirajuću ulogu ima žderanje prostirke, sa posledičnim nepotpunim opstrukcijama digestivnog kanala, kao i ishrana zrnevljem žitarica bez dodatka peska i šljunka. Bolesti druge etiologije stvaraju pogodne uslove za razmnožavanje i patogeno delovanje klostridija. Značaj se veliki pridaje supkliničkoj kokcidiozi, ali ulogu imaju i Gamboro bolest i zarazna anemija pilića.

Poseban značaj u širenju i održavanju bolesti ima biološka karakteristika klostridija da stvaraju vrlo otporne spore, koje dugo preživljavaju nepovoljne uslove spoljašnje sredine, a otporne su i na većinu dezinficijenasa.

Predmet istraživanja je bio da se kroz kompleks enteritisa u živinarskoj proizvodnji sagleda učestalost predisponirajućih faktora na pojavu klostridijalnih infekcija u jatima živine sa ciljem njihovog ublažavanja, odnosno otklanjanja.

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UNEXPECTED SEROLOGICAL FINDINGS IN BREEDERS VACCINATED AGAINST NON-TYPHOID *SALMONELLA*

D. Potkonjak[1], M. Velhner[1], E. Nikolić Đorić[2], M. Kapetanov[1], D. Orlić[1], A. Potkonjak[3], B. Lako[3]

[1]Scientific Veterinary Institute Novi Sad, Rumenački put 20, 21000 Novi Sad, Serbia
[2]University of Novi Sad, Faculty of Agriculture Novi Sad, Department of Agriculture Economics and Rural Sociology, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia
[3]University of Novi Sad, Faculty of Agriculture Novi Sad, Department of Veterinary Medicine, Trg Dositeja Obradovića 8, 21000 Novi Sad, Serbia
Corresponding author: dpotkonjak@niv.ns.ac.rs
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Abstract: On a rearing poultry farm, situated at northern part of Serbia, vaccination in two commercial breeder flocks with inactivated vaccine against *Salmonella Enteritidis* was conducted. During rearing, vaccines against major poultry pathogens were administered and additionally, inactivated *Salmonella Enteritidis* bacterin at age of 6, 12 and 16 to 18 weeks. Efficacy of immunization protocol was tested applying commercial ELISA before and after vaccinations. Three weeks after the vaccination, at age of 9 weeks, antibodies against salmonella were detected in both breeder flocks. At 21 weeks of age, seroconversion attributed to salmonella vaccine was at low rate in Ross breeders (30 to 45%) and in egg-type Hy-Line breeders very low (20%). Different antibody level and dynamics of humoral response were found upon comparison hybrids and gender. Evaluation of immunization success during rearing on farm by serological screening was hard to achieve. Nevertheless, due to prolonged stimulation of humoral immunity, further use of inactivated vaccines is inevitable.

Key words: breeder chickens, non-typhoid salmonellae, vaccination, monitoring

Introduction

Poultry products or poultry itself are important source of non-typhoid salmonellae, particularly of serotypes that are frequently responsible for disease in humans (*EFSA, 2006*; *EFSA, 2007*; *Hrnjaković Cvjetković et al., 2011*). An overall decrease in number of reported salmonellosis outbreaks is attributed to significant

reductions in commercial poultry due to effective national control programs targeting against salmonellae that are of public concern (*EFSA, 2012*). The use of live and inactivated salmonella vaccines are reviewed (*EFSA, 2004*), and vaccination is conducted in most of European countries. Good farm management is extremely important in reducing or eliminating infectious agents in closed farming while vaccination against salmonella additionally contributes to decrease shedding and improve food safety. Considering the shortcomings in poultry industry in Serbia and need for substantial improvements in management it is our belief that vaccination against salmonellae has to be introduced on regular basis in breeder flocks. It was not long ago when serology monitoring was introduced in diagnostic practice in Serbia, to determine flock status regarding salmonella. Older flocks in production either vaccinated or not, often tested serologically positive, while during rearing negative results were found in vaccinated breeders (*Velhner et al., 2005*; *Potkonjak et al., 2007*). Thus immunization of breeders on a farm with good management and biosafety was monitored with ELISA to estimate the seroconversion rate and level of serum antibodies and try to get insight into vaccination efficacy.

Material and Methods

Rearing farm

On-farm monitoring was performed in two commercial parent flocks of meat- and egg-type, of Ross and Hy-Line provenience, respectively, settled on one rearing farm. The farm was physically isolated with wired fence and located aside the main roads. Rigorous control at entry, disinfection barrier, changing rooms and toilets, overshoes, were all provided. Visitors could be easily differentiated from farm personnel by the color of clothes. Cleaning and disinfection of poultry houses, equipment and on farm traffic, were readily done, using commercial disinfectants and fumigation with formalin. The farm rested for more than three weeks. Houses were fully equipped with line feeders and drinkers, automated control systems for air conditioning and heating. Separate cockerels and pullets rearing on floor system with straw bedding were done according to technological guidelines.

Chickens and vaccination program

One day old breeder chickens of provenience Ross 308 (meat-type) and Hy-Line brown (egg-type) in total quantity of approximately 28000 and 16000, respectively, were housed separately by gender. Complete mashed diets without antibiotics and anticoccidials were provided throughout rearing period, and 0.1% of acetic acid was added in drinking water on a daily basis.

Vaccination against salmonellosis was performed by intramuscular injection of commercial inactivated vaccine (*Nobilis Salenvac®,Intervet*) in breast muscle (0.5ml vaccine/bird) at 6, 12 and 16 to 18 weeks of age, according to producer's recommendations. Immunization protocol also included major poultry pathogens, e.i. Marek's disease, coccidiosis, infectious bronchitis, Newcastle disease, infectious bursal disease, REO virus, avian pox, using attenuated and inactivated vaccine strains.

Sera collection

Blood was taken from healthy birds by venepuncture. Approximately 2-3 ml of individual blood samples (twenty per flock) were collected on farm, before vaccination (at 6 weeks of age) and at age of 8, 9, 11, 14, 15 and 21 weeks. Sera were separated and kept in refrigerator until testing, not longer than 24 hours.

Serology

For serological screening, commercial ELISA test (*Flockscreen* ^{TM}, Guildhay, Biomedica gruppe) was performed according to instructions provided by manufacturer. This ELISA contains LPS of *S. Enteritidis*. Absorbance values determined at 500nm, were used to calculate S/P ratio and antibody titer. Interpretation of the results is given in Table1.

Table1. *Salmonella Enteritidis* (SE) status interpretations based on ELISA testing

S/P	*Antibody titer to SE*	**SE status**
≤ 0.08	0 - 450	negative
0.08 - 0.25	451 -1500	suspect
> 0.25	≥ 1501	positive

Statistical analysis

All obtained data were analyzed with descriptive statistics. Due to some untypical and extreme observations, other tests were used, too. To determine the difference of response to salmonella vaccination on flock level and varriations in and between flocks, tests of *Kruskal Walis* and *Mann-Whitney U* were used. The course of average antibody titer through selected week periods was rated using linear regression method of the least square and robust regression method *(Rousseeuw , 1990; Dorfman, 1996),* with software *S-PLUS Professional Realise 1, Mathsoft Inc.*

Results and Discussion

Immunization of poultry against non-typhoid salmonellae is useful tool to reduce its overall prevalence and increase resistance to infection with wild strains. Several field studies have confirmed beneficial effects of salmonella vaccines (*Oostenbach, 1998*; *Feberwee et al., 2001*; *Davies and Breslin, 2003*; *Groves and Pavic, 2005*) and serological monitoring is used during rearing and exploitation (*Wierup et al., 1995*; *Hoop, 1997*; *Wegener et al, 2003*; *Potkonjak, 2009*). In attempt to monitor serological status of two breeder flocks that were vaccinated under field conditions with commercial inactivated salmonella vaccine, sera were tested before and after immunization. Detectable individual SE antibody titers were found in hens of both breeder flocks three weeks after the vaccination at age of 9 weeks (Table 2). However, low mean antibody titers were found at age 6, 8 and 11 weeks, in Ross and Hy-Line breeders. At 6 weeks of age in the absence of maternal antibodies, there was no indication of recent infection. At 8 and 11 weeks, time points representing two and five weeks after prime vaccination indicate that humoral response is short-lived and transient. According to published trials (*Clifton-Hadley et al., 2002*; *Atterbury et al., 2010*) inactivated vaccines need to be administered twice or combined with live strains. On the other hand, infection of chickens at early age provokes antibody response (*Cooper et al., 1989*; *Desmidt et al., 1996*; *Davies et al., 1997*; *Sasai et al., 1997*; *Skov et al., 2002*; *Babu et al., 2004*). After age of 11 weeks mean antibody titer tend to increase, but with higher numerical variations. At week 14 (2 weeks after second vaccination), only in pullets of Ross provenience, mean titer indicated positive status, whilst in Ross cockerels and Hy-Line pullets and cockerels, result was rather suspect, according to manufacturer interpretation. Consecutive vaccination with inactivated vaccines only or as buster to live strains, showed triggering protective responses in commercial poultry (*Woodward et al., 2002*; *Atterbury et al., 2010*). In a study of *McMullin et al.* (1997) low antibody level and less positive sera were determined in 14 weeks old commercial flocks vaccinated twice (at age of 11 to 12 and 15 to 16 weeks, respectively). In our work, detail analysis of the titer distribution for each flock (data not shown) revealed significant difference due to some individual extremes as indicated on Fig.1 and Fig.2. At age of 14 weeks, an increase in mean antibody titer was observed, albeit reached positive value only in meat-type pullets and remained at slightly higher level at week 15 (Table 2, Table 3). In contrast, significant decrease of mean antibody level was found at 15 weeks of age in egg-type breeders. Reasons for such fluctuations remain unclear. Killed vaccine induced elevated serum antibodies in mature hens that were vaccinated once (*Okamura et al., 2007*). It was speculated that technology and management such as stress in period of feed restriction may cause temporary poor response to vaccine

(*McMullin et al., 1997*). At age of 21 weeks (3 to 5 weeks after the third vaccination), unexpectedly low mean SE titer and low number of positive sera were detected in all flocks, applying commercial ELISA test. Moreover, all sera from Hy-Line cockerels were individually tested either negative or suspect. The farm has trained and experienced stuff, however technical issues remain major disadvantage of vaccines that are administrated parenterally. Perhaps if number of sera was higher, more conclusive results would be obtained. Some control programs recommend 60 samples per flock to be taken for serology, and use of gm flaggelin as well (*Yap et al., 2001*; *Potkonjak, 2009*). Another observation was that in both hybrids positive sera were found more profound in females in compare to males. At age of 14 and 21 weeks mean antibody titer to *S. Enteritidis* was significantly higher in females of Ross in compare to Hy-Line (Table 3, Table 4). *Liu et al.* (2003) found significant difference in response to vaccine in selected chicken lines, which may partially explain results obtained in this research. Host resistance to salmonella is controlled by genetic factors and because of that, identification of genes important for host resistance (*Lamont et al., 2002*; *Malek et al., 2004*) would contribute selection of lines with enhanced protection against non-typhoid salmonella.

Table 2. Results of serological testing in meat- and egg-type breeders prior and after vaccinations

Age (week)	Ross			Hy-Line		
	♂	♀	overall	♂	♀	overall
6	-*	-	-	-	-	-
8	-	-	-	-	-	-
9	-	1/20 (5%)	1/20	-	1/20 (5%)	1/20
11	-	-	-	-	-	-
14	5/20 (25 %)	9/20 (45 %)	14/40	4/20 (20%)	4/20 (20%)	8/40
15	2/20 (10%)	6/20 (30%)	8/40	2/20 (10%)	6/20 (30%)	8/40
21	6/20 (30%)	9/20 (45%)	15/40	0/20 (0%)	4/20 (20%)	4/40

* - negative in ELISA

Table 3. Mean antibody titer to SE in Ross and Hy-Line breeders.

Age (weeks)	Mean titer	Median	Standard deviation	Variation Coefficient (%)	Mean titer	Median	Standard deviation	Variation Coefficient (%)
♂	Ross				Hy-Line			
6	90.500	112.00	69.665	76.98	267.100	0.00	1139.074	426.46
8	11.000	0.00	21.121	192.01	129.950	0.00	250.736	192.95
9	426.700	495.00	255.680	59.92	509.950	448.00	319.444	62.64
11	87.900	44.50	129.949	147.84	162.25	135.00	168.60	103.91
14	*720.900	94.00	1231.829	170.87	*1393.850	942.50	1817.683	130.41
15	486.150	186.50	729.877	150.13	*839.150	495.50	1281.897	152.76
21	*32.550	4.50	51.218	157.35	*1273.650	1031.50	1186.202	93.13
♀								
6	232.000	173.50	212.769	91.71	188.350	142.00	218.175	115.83
8	6.600	0,00	17.169	260.14	134.600	38.00	173.516	128.91
9	681.450	384.50	1205.390	176.89	267.100	0.00	1139.074	426.46
11	84.350	21.00	145.969	173.05	129.950	0.00	250.736	192.95
14	1539.400	523.00	2486.304	161.51	509.950	448.00	319.444	62.64
15	3606.000	349.50	6160.161	170.83	*162.25	135.00	168.60	103.91
21	1473.650	147.50	4535.331	307.76	1393.850	942.50	1817.683	130.41

*- $p<0.01$

Table 4. Results of *S. Enteritidis* antibody titer comparison in pullets and cockerels of different hybrid with *Kruskal Walis* test

Week	6	8	9	11	14	15	21
Cockerels	13,18377** p =0,0003	3,035969 p =0,0814	0,0090125 p =0,9244	21,89744** p =0,0000	5,297020* p =,0214	1,695876 p =0,1928	25,29471** p =0,0000
Pullets	1,181594 p =0,2770	6,456286* p =0,0111	0,0737379 p =0,7860	22,49717** p =0,0000	1,292065 p =,2557	1,171171 p =0,2792	12,01309** p =0,0005
Flocks	9,203972** p =0,0024	9,316420** p =0,0023	0,1309111 p =0,7175	44,70991** p =0,0000	6,023516* p =0,0141	3,191596 p =0,0740	34,50640** p =0,0000

*, ** - indices of significance, $p<0.1$ and $p<0.01$

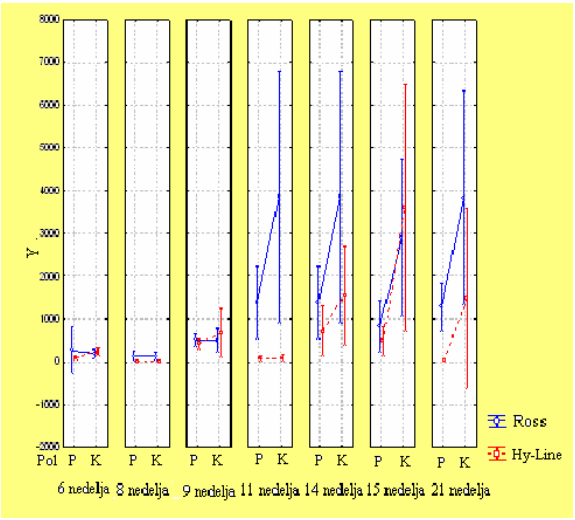

Figure 1. Medians *S. Enteritidis* antibody titer in weeks for Ross and Hy-Line breeder flocks (95% confidence interval)

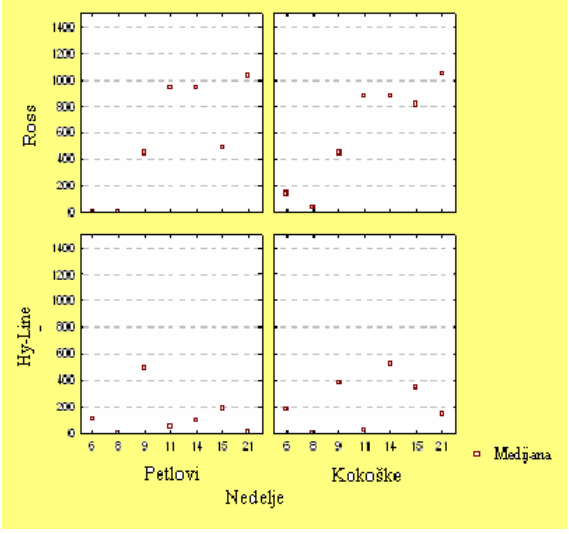

Figure 2. Distribution of medians of *S. Enteritidis* antibody titer in breeder flocks separately presented for cockerels and pullets (in selected weeks).

Conclusion

Serological screening of breeders during rearing showed inconsistent humoral response to inactivated vaccine against *Salmonella Enteritidis*. Comparison between two hybrids and genders revealed differences in antibody level and dynamics of humoral immunity. Evaluation of immunization success during rearing on farm by serological screening seems to be a difficult task. However, due to prolonged stimulation of humoral immunity, further use of inactivated vaccines is inevitable.

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Neočekivani serološki nalazi u roditeljskim jatima vakcinisanih protiv *Salmonella enteritidis*

D. Potkonjak, M. Velhner, E. Nikolić Đorić, M. Kapetanov, D. Orlić, A. Potkonjak, B. Lako

Rezime

Vakcinacija dva komercijalna roditeljska jata protiv *Salmonella* Enteritidis je sprovedena u toku odgoja na farmi lociranoj u južnobačkom okrugu. U toku odgoja primenjene su vakcine protiv bolesti živine, i dodatno protiv salmoneloze, inaktivisanom *Salmonella Enteritidis* vakcinom u uzrastu od of 6, 12 i 16 do 18 nedelja. Efikasnost protokola imunizacije je ispitana primenom komercijalnog ELISA testa pre i posle vakcinacije. Tri nedelje posle vakcinacije, u uzrastu od 9 nedelja, detektovana su serumska antitela na salmonele u oba jata. Niska i vrlo niska serokonverzija utvrđena je kod roditelja Ross provinijencije (30 do 45%) i Hy-Line roditelja (20%), u uzrastu od 21 nedelje. Nakon poređenja dva tipa linijskih hibrida i polova, primećene su razlike u pogledu nivoa antitela i dinamike humoralnog odgovora. Veoma je teško da se proceni uspeh imunizacije u toku odgoja na farmi, serološkim skriningom. Uprkos tome, zbog duže stimulacije humoralnog imuniteta, dalja upotreba inaktivisanih vakcina je neizbežna.

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EFFECT OF PROTEASE ON MEAT YIELD OF BROILERS

V. Dosković[1], S. Bogosavljević-Bošković[1], Z. Pavlovski[2], B. Milošević[3], Z. Škrbić[2], S. Rakonjac[1], V. Petričević[2]

[1]Faculty of Agronomy, University of Kragujevac, Cara Dušana 34, 32000 Čačak,
[2] Institute for Animal Husbandry, Autoput 16, P. Fah 23, 11080, Belgrade-Zemun
[3] Faculty of Agriculture, University of Priština, Kosovska Mitrovica, 38219 Lešak
Corresponding author: vladosko@kg.ac.rs
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Abstract: This study reports the effect of protease and reduced crude protein content on carcass weight and dressing percentage in fast-growing Cobb 500 broilers. The length of fattening was 49 days. E-I and E-II experimental broilers were fed complete feeds containing 0.2 and 0.3% (Ronozyme Pro Act) protease supplementation and crude protein levels reduced by 4% and 6%, respectively, compared to control broilers (C). Performance traits were evaluated. At the end of the fattening trial on day 49, 10 male and 10 female birds were randomly sacrificed from each experimental group to determine body weight, conventionally dressed, ready-to-roast and ready-to-grill carcass weights, and abdominal fat weight. The results suggest no significant effect of dietary treatments on carcass weight, dressing percentage, weight and abdominal fat content. Significant differences were observed in carcass weight and carcass yield between female and male broilers, whereas broiler sex had no significant effect on abdominal fat percentage.

Keywords: broilers, protease, dressing percentage.

Introduction

Modern chicken meat production involves the use of fast-growing strains and diet formulations that allow them to express their maximum genetic potential. Major characteristics of modern broiler strains include fast growth rates, high breast and leg muscle weight, and relative inactivity or poor mobility *(Gous and Cherry, 2004)*. However, to boost profitability, the broiler industry is steadily pushing broilers beyond their optimum biological limits through reduced fattening periods, increased final body weights, changes in body conformation, higher breast percentage; the use of feed additives in broiler diets and the administration of different products for disease prevention and control.

Poultry meat quality traits are affected by a range of factors such as strain, rearing method, nutrition, biologically active substances, health, welfare and the environment, which have a significant impact on muscle metabolism and meat chemical composition. Maize-soya based broiler diets are used in modern poultry production, with problems regarding GMO contamination of organic feed leading to research on the potential substitution of soybean meal as a source of protein *(Meluzzi et al., 2009)*. Broiler nutrition issues offer a challenge to nutritionists, demanding continuous dietary modifications, primarily in terms of the increased use of biologically active substances (enzymes, antioxidants, organic acids, etc.). The use of dietary enzymes is nutritionally, economically and environmentally justified. Numerous studies have shown that enzyme supplementation of broiler diets has no adverse effect on body weight, feed intake, feed conversion, nutrient digestibility, meat quality and production costs. One of the most important nutritional requirements for optimum animal performance is to ensure an adequate dietary protein level *(Bregendahl et al., 2002; Wijtten et al., 2004)*, given the vast array of functions performed by proteins in living organisms. Moreover, some researchers have dealt with a potential reduction in dietary protein levels *(Rostagno et al., 2007; Horniakova and Abas, 2009)* to help reduce nitrogen excretion into the environment *(Aletor et al., 2000)* as well as feeding costs, in view of the fact that protein feeds as sources of protein entail great expenses.

The objective of this study was to determine the effect of reduced crude protein levels in protease-supplemented diets on broiler meat yield.

Material and Methods

A total of 300 day-old Cobb 500 broilers were allocated to three groups-boxes each containing 100 birds at a stocking density of 10 birds/m^2.

The broilers were randomly grouped, giving a random ratio of male to female birds across groups. Chicks had free access to water and feed, and a 24-h photoschedule was applied. Ad libitum feeding was used.

A three-stage feeding-fattening program was used, including starter (0-21 days), grower (22-42 days) and finisher stages (42-49 days).

One group of broilers served as the control-C (fed normal nutrient levels, in accordance with broiler requirements during certain fattening stages), and the other two groups were experimental groups E-I and E-II (fed diets containing 4% and 6% lower crude protein levels and supplemented with 0.2% and 0.3% protease, respectively).
Broiler feeds were in powdered form. Feed ingredients (used across fattening stages and test groups) and the chemical composition of feeds are presented in Table 1.

A protease preparation commercially called Ronozyme ProAct (produced by DSM, The Netherlands) was used in the fattening trial. The product is intended for use as a feed additive in chickens for fattening at a recommended dose of 200 mg kg-1.

Table 1. Ingredients and nutrient composition of experimental diets for fattening chickens[1]

Ingredient, %	Starter stage (1 to 21 d)			Grower stage (22 to 42 d)			Finisher stage (43 to 49 d)		
Treatments	C	E-1	E-2	C	E-1	E-2	C	E-1	E-2
Maize	52.49	54.92	56.26	63.15	65.28	66.34	68.62	70.60	71.59
Soybean meal	22.24	19.79	18.44	13.00	10.85	9.78	9.10	7.10	6.10
Soybean groats	18.50	18.50	18.50	17.00	17.00	17.00	15.40	15.40	15.40
Feeding yeast	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
L-Lysine (78%)	0.10	0.10	0.10	0.20	0.20	0.20	0.23	0.23	0.23
DL-Methionine (99%)	0.22	0.22	0.22	0.30	0.30	0.30	0.30	0.30	0.30
Limestone	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Monocalcium phosphate	1.30	1.30	1.30	1.20	1.20	1.20	1.20	1.20	1.20
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Calcium formiate (30.5%)	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Captex T	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Premix	1	1	1	1	1	1	1	1	1
Protease	0.00	0.20	0.30	0.00	0.20	0.30	0.00	0.20	0.30
Calculated composition									
ME, kcal/kg	3.081	3.100	3.112	3.157	3.174	3.183	3.181	3.198	3.207
Crude proteins, %	22.59	21.72	21.24	18.99	18.22	17.84	17.16	16.45	16.09
Crude fats, %	5.59	5.55	5.70	5.67	5.73	5.76	5.55	5.61	5.64
Ca, %	0.96	0.95	0.95	0.91	0.91	0.90	0.90	0.89	0.89
Total P, %	0.73	0.72	0.72	0.68	0.67	0.67	0.66	0.65	0.65
Available P, %	0.44	0.44	0.43	0.40	0.40	0,40	0.39	0.39	0.39
Total lysine, %	1.33	1.27	1.24	1.15	1.10	1.08	1.05	1.00	0.98
Methionine+cystine, %	0.92	0.90	0.89	0.91	0.89	0.88	0.86	0.84	0.83

[1] Treatments: C-control group, standard broiler diet, without protease; E-I- broilers fed a diet with a 4% reduction in crude protein level as compared to the control group, and 0.2% protease supplementation; E-II broilers fed a diet with a 6% reduction in crude protein level as compared to the control group, and 0.3% protease supplementation.

On day 49 of the fattening trial, 10 male and 10 female broilers were randomly selected from each group, tagged, weighed and slaughtered after a fasting period of 10 hours.

At slaughter, conventionally dressed, ready-to-roast and ready-to-grill carcass weights and abdominal fat weight were determined and used to calculate dressing percentage and pre-slaughter abdominal fat percentage.

The data were subjected to conventional statistical methods. The significance of differences was tested by a two-factor analysis of variance using a 3x2 design (3 feeding treatments and 2 sexes).

The significant differences determined by the analysis of variance and results of F-exp values were evaluated using Tukey's test. Significance was accepted at $P<0.05$. The test parameters were subjected to an analysis of variance using ANOVA, *Microsoft STATISTICA Ver. 5.0, StatSoft Inc. (1995)*.

Results and Discussion

Table 2. presents conventionally dressed, ready-to-roast and ready-to-grill carcass weights and abdominal fat weight in broilers.

Table 2. Dressed carcass yield and abdominal fat weight of broilers (in g)

Groups	Sex		Pre-slaughter body weight	Conventionally dressed carcass weight	Ready-to-roast carcass weight	Ready-to-grill carcass weight	Abd. fat weight
C (no protease)	♂	$\bar{x}$	3463.50	2986.78[a]	2807.19[a]	2581.70[a]	50.06
		Sd	104.48	100.74	94.43	88.24	13.62
	♀	$\bar{x}$	2898.50	2520.07[b]	2386.67[b]	2176.15[b]	49.43
		Sd	160.68	156.79	146.44	130.54	12.39
	♂+♀	$\bar{x}$	**3181.00**	**2753.42**	**2596.93**	**2378.92**	**49.74**
		Sd	**318.44**	**271.61**	**246.81**	**234.61**	**12.68**
E-I (0.2% protease)	♂	$\bar{x}$	3394.00	2916.37[a]	2738.01[a]	2501.72[a]	57.31
		Sd	133.35	125.25	120.28	123.71	18.17
	♀	$\bar{x}$	2877.50	2519.08[b]	2381.65[b]	2170.70[b]	49.89
		Sd	114.19	109.13	106.15	106.31	11.79
	♂+♀	$\bar{x}$	**3135.75**	**2717.72**	**2559.83**	**2336.21**	**53.60**
		Sd	**291.21**	**233.69**	**213.56**	**203.56**	**15.39**
E-II (0.3% protease)	♂	$\bar{x}$	3388.50	2909.46[a]	2729.95[a]	2510.34[a]	48.97
		Sd	205.25	179.10	167.95	161.24	11.20
	♀	$\bar{x}$	2816.50	2442.02[b]	2302.95[b]	2096.93[b]	48.37
		Sd	78.35	69.44	71.68	59.39	14.97
	♂+♀	$\bar{x}$	**3102.50**	**2675.74**	**2516.45**	**2303.63**	**48.67**
		Sd	**330.10**	**273.82**	**252.54**	**242.82**	**12.87**
p-value							
Source of variation							
Protease			0.210	0.170	0.123	0.131	0.503
Sex			0.001	0.001	0.001	0.001	0.425
Protease x sex			0.790	0.615	0.602	0.470	0.672

[a-b] Means with different superscripts within columns differ significantly ($P<0.05$)

The data in Table 2. reveal similar carcass weights, regardless of dietary treatment; therefore, no significant effect (P>0.05) of diet on the carcass quality traits tested was observed. In addition, broilers had similar abdominal body weight, with no difference observed (P>0.05). Pre-slaughter body weight was lower in female birds than in males; hence, dressed carcass weight was significantly lower in females, compared to male broilers (P<0.05), whereas abdominal fat weight showed no significant differences between the sexes (P>0.05).

Given the above-mentioned results, dressed carcass yield was largely dependent on slaughter weight, with the average dressed carcass weights being higher in broilers having increased average slaughter weights.

Data on the dressing percentage of dressed carcass weights and pre-slaughter abdominal fat percentage, across test factors (dietary treatments, broiler sex), are given in Table 3.

Table 3. Dressing percentage and abdominal fat percentage (in %)

Groups	Sex		Dressing percentage for conventionally dressed carcass	Dressing percentage for ready-to-roast carcass	Dressing percentage for ready-to-grill carcass	% abd. fat
C (no protease)	♂	$\bar{x}$	86.23^{b}	81.05^{bc}	74.54^{abc}	1.44
		Sd	0.87	0.92	1.08	0.38
	♀	$\bar{x}$	86.92^{ab}	82.32^{a}	75.06^{ab}	1.70
		Sd	0.87	0.85	0.65	0.40
	♂+♀	$\bar{x}$	**86.57**	**81.68**	**74.80**	**1.57**
		Sd	**0.91**	**1.08**	**0.91**	**0.40**
E-I (0.2% protease)	♂	$\bar{x}$	85.92^{b}	80.66^{bc}	73.69^{c}	1.69
		Sd	0.65	0.85	1.6	0.56
	♀	$\bar{x}$	87.53^{a}	82.76^{a}	75.42^{a}	1.74
		Sd	0.63	0. 90	1.24	0.45
	♂+♀	$\bar{x}$	**86.73**	**81.71**	**74.55**	**1.72**
		Sd	**1.04**	**1.37**	**1.43**	**0.50**
E-II (0.3% protease)	♂	$\bar{x}$	85.86^{b}	80.56^{c}	74.07^{bc}	1.45
		Sd	0.59	0.72	0.86	0.34
	♀	$\bar{x}$	86.71^{ab}	81.76^{ab}	74.45^{abc}	1.71
		Sd	0.90	0.95	0.81	0.48
	♂+♀	$\bar{x}$	**86.28**	**81.16**	**74.26**	**1.58**
		Sd	**0.86**	**1.03**	**0.84**	**0.43**
p-value						
Source of variation						
Protease			0.131	0.186	0.227	0.504
Sex			0.001	0.001	0.001	0.110
Protease x sex			0.470	0.128	0.063	0.687

[a-c] Means with different superscripts within columns differ significantly (P<0.05)

The dressing percentage of conventionally dressed carcass and ready-to-roast carcass was highest in E-I broilers (86.73% and 81.71%) and lowest in E-II broilers (86.28% and 81.16%, respectively). E-II was also found to have the lowest dressing percentage of ready-to-grill carcass (74.26%), which was the highest in control broilers (74.80%). Pre-slaughter abdominal fat percentage was 1.57-1.72%. As regards the effect of dietary treatments on dressing percentage, no significant effect of this factor on the percentage of differently dressed carcasses in liveweight was observed ($P>0.05$). In contrast, broiler sex was found to affect the dressing percentage of broilers, with E-I females exhibiting significantly higher values for conventionally dressed and ready-to-grill carcasses, compared to male birds ($P<0.05$). Moreover, the dressing percentage of ready-to-roast carcass was higher in female broilers than in males, with the significance observed in all three groups ($P<0.05$). Pre-slaughter abdominal fat percentage was not affected by either dietary treatment or broiler sex ($P>0.05$).

The comparison of the present results to those reported by other authors suggests that the values for the dressing percentage of both conventionally dressed and ready-to-roast carcass were somewhat higher than those obtained by *Nikolova et al. (2011)* in a 49-day fattening trial. Also, the dressing percentage of ready-to-grill carcass was somewhat higher than the values reported by *Café et al. (2002)*, *Si et al. (2004)*, *Abudabos (2010)*, *Bogosavljević-Bošković et al. (2011a)*, *Nikolova and Bogosavljević-Bošković (2011)*, *Petričević et al. (2011),* etc. Differences in dressing percentage values may be due to differences in the length of fattening stages, stocking density, …

Furthermore, dressed carcass yield and dressing percentage were not significantly affected by dietary treatments, which is in agreement with the results obtained by *Espino et al. (2000)*, *Osei and Oduro (2000)*, *Café et al. (2002)*, *Yadav and Sah (2005)*, *Abudabos (2010)* and *Frietas et al. (2011)*. Conversely, *Hajati et al. (2009)* reported an increase in dressing percentage due to the effect of enzymes. Broiler sex was found to have a significant effect on ready-to-grill carcass weight and no effect on dressing percentage (*Bogosavljević-Bošković et al., 2011a*). *Bogosavljević-Bošković et al. (2011b)* found a somewhat higher dressing percentage of conventionally dressed, ready-to-roast and ready-to-grill carcasses in females than in males, with the effect of sex being significant only for the dressing percentage of ready-to-roast carcass ($P<0.05$).

Pre-slaughter abdominal fat percentage was considerably lower compared to the results on Cobb 500 broilers at 49 days reported by *Café et al. (2002)*, *Si et al. (2004), Ahmadi and Karimov (2010)*, as well as to the findings of *Hajati et al. (2009)* for broilers at 44 days. The values for pre-slaughter abdominal fat percentage were not affected by dietary treatments, which complies with the results of *Hajati et al. (2009)* in their study on an enzyme complex containing arabinoxylanase and β-glucanase, *Abudabos (2010)* who used an enzyme complex (β-pentosanase, α-

amilase, glucanase and galactomanase), *Frietas et al. (2011)* who studied the effect of protease. In contrast, *Café et al. (2002)* found a significantly higher abdominal fat content in broilers fed diets supplemented with an enzyme complex containing xylanase, protease and amylase.

Conclusion

The results obtained suggest that the test carcass quality parameters were much more affected by broiler sex than by dietary treatments; moreover, the reduction in crude protein level in broiler feeds supplemented with 0.2 and 0.3% protease had no effect on dressed carcass weight, dressing percentage of conventionally dressed, ready-to-roast and ready-to-grill carcasses, and weight and pre-slaughter percentage of abdominal fat. Compared to female broilers, male birds had significantly higher dressed carcass weights and significantly lower dressing percentages. No significant effect of broiler sex on abdominal fat weight and percentage was observed.

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Uticaj proteaze na prinos mesa tovnih pilića

V. Dosković, Snežana Bogosavljević-Bošković, Z. Pavlovski, B. Milošević, Zdenka Škrbić, S.Rakonjac, V. Petričević

Rezime

U radu je analiziran efekat enzima proteaze, uz snižen nivo sirovih proteina na masu i udeo različito obrađenih trupova pilića brzorastućeg hibrida-Cobb 500. Tov pilića trajao je 49 dana. Ogledne grupe pilića E-I i E-II hranjene su potpunim smešama za tov pilića koje su imale za 4, odnosno za 6% manje sirovih proteina u odnosu na kontrolnu grupu (C), uz dodatak 0,2, odnosno 0,3% enzima proteaze (Ronozyme Pro Act) u hranu. U toku ogleda praćene su proizvodne osobine. Na kraju ogleda 49.dana, slučajnim izborom, odabrano je iz svake ogledne grupe po 10 muških i ženskih grla za klanje, izmerena njihova telesna masa, a na liniji klanja i masa klasično-obrađenog trupa, trupa „spremno za pečenje“, trupa „spremno za roštilj“ i udeo abdominalne masti.

Na osnovu rezultata istraživanja zaključeno je da nije bilo signifikantnog uticaja različitih formulacija hrane na masu i randman trupova i masu i udeo abdominalne masti. Istovremeno, između kokica i petlića pojavile su se značajne razlike u masi i prinosu trupova pilića, dok nije bilo razlika u sadržaju abdominalne masti pod uticajem pola pilića.

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MYCOLOGICAL DETOXIFICATION OF AFLATOXIN B_1 AND OCHRATOXIN A *IN VITRO*

A. Bočarov-Stančić[1], J. Lević[2], M. Bodroža-Solarov[3], V. Pantić[1], N. Salma[1], J. Vučković[3]

[1]Bio-Ecological Center DOO, Petra Drapšina 15, 23000 Zrenjanin, Republic of Serbia
[2]Maize Research Institute Zemun Polje, Slobodana Bajića 1, 11185 Belgrade-Zemun, Republic of Serbia
[3]Institute for Food Technology, Bul. Cara Lazara 1, 21000 Novi Sad, Republic of Serbia
Corresponding author: naukabec@bioec.rs
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Abstract: The ability of 37 and 45 atoxigenic fungal isolates to detoxificate aflatoxin B_1 ($AFLB_1$) and ochratoxin A (OTA), respectively, was investigated under controlled laboratory conditions. Crude extract of mycotoxin was added to the test medium (modified Vogel's medium) to the final concentrations of 0.6 μg mL^{-1} ($AFLB_1$) and 10.0 μg mL^{-1} (OTA). The presence of mycotoxin residue in the test medium was determined after 7 and 14 days of fungal incubation on test medium at 26±1 °C. Majority of Aspergilli (1/2 *A. clavatus*, 2/5 *A. flavus*, 5/7 *A. fumigatus*, 1/1 *A. nidulans*, 8/10 *A. niger*, 1/1 *A. terreus* and 2/3 *Aspergillus* spp.) reduced the concentration of $AFLB_1$ from 20.0 to 85.0%. The highest detoxification of $AFLB_1$ (95.0%) was demonstrated *in vitro* by two fungal species: *Cladosporium* sp. and C*ephaliophora tropica*. The total of 28.89% fungal isolates was able to reduce the OTA concentration *in vitro.* Isolates of *A. fumigatus* (57.1%), *A. niger* (41.7%) and *R. stolonifer* (15.4%) detoxificated 10.0-70.0%, 10.0-50.0% and 30.0-50.0% of applied OTA, respectively. *Trichoderma* sp. and *Purpureocillium lilacinus* were the most effective fungal isolates in the reduction of the presence of this mycotoxin (80.0% and 90.0%, respectively). Obtained results indicate that one isolate of A. niger (47-2/10) is the most promising candidate for environmental remediation and as feed additive because it detoxificated both $AFLB_1$ and OTA.

Key words: aflatoxin B1, ochratoxin A, mycological detoxification, in vitro

Introduction

Aflatoxin B1 ($AFLB_1$) is one of the most harmful toxic naturally occurring mycotoxins, because of its hepatotoxic, teratogenic, immunosuppressive and mutagenic nature. The International Agency for Research on Cancer classified this difuranocoumarocyclopentanone in a group I as human carcinogen *(IARC, 2002)*. Due to the ubiquitous occurrence of $AFLB_1$, produced by fungal species *Aspergillus flavus* Link ex Fr., *A. parasiticus* Speare and *A. nomius* Kurtz *(Bakutis et al., 2005),* on cereal grains, oil seeds, nuts, spices, milk etc., preventive and remedial measures are necessary, including detoxification procedures.

Ochratoxin A (OTA), is also considered to be one of the most toxic naturally occurring mycotoxins, whose presence has been establish in food, feed and commercial feed mixtures. In order to reduce the presence of this dihydro-isocoumarin coupled to β-phenylalanine, different methods for the inhibition of the growth of fungi producing OTA (*Aspergillus alliaceus* Thom & Church*, A. carbonarius* (Bain.) Thom*, A. ochraceus* Wilhelm*, A. steynii* Fris. & Samson*, A. westerdijkiae* Fris. & Samson*, Penicillium nordicum* Dragoni & Cantoni and *P. verrucosum* Eierchx) and for the prevention of this mycotoxin production are applied *(Abrunhosa et al., 2010)*.

Biological methods e.g. detoxification of $AFLB_1$ and OTA, either by the whole cell or an enzyme system have been considered increasingly as an alternative to physical and chemical treatments. Numerous microorganisms capable of degrading, absorbing and detoxifying these mycotoxins are reported in the literature. Beside several protozoan species, yeasts and bacteria *(Hwang and Draughon, 1994, Piotrowska and Žakowska, 2000)*, it is considered that filamentous fungi also have these capabilities. According to literature data following fungi play very important role in reduction of concentration of $AFLB_1$ and OTA: Zygomycota (*Rhizopus* spp. and *Mucor* spp.), Ascomycota (*A. flavus, A. fumigatus* Fresen., *A. niger* (Fr.) P. Karst*, A. ochraceus, A. parasiticus, A. wentii* Wehmer, *Neurospora* sp. and *Trichoderma viride* Pers.), plant pathogens (*Botrytis cinerea* Pers., *Peniophora* sp.*, Phoma* sp. and *Alternaria* sp.) as well as Basidiomycota (*Armillariella tabescens* (Scop.) Emel, *Pleurotus ostreatus* Jacq. and other white rot fungi) *(Shapira and Paster, 2004; Alberts, 2007; Alberts et al., 2009; Abrunhosa et al., 2010)*.

Detoxification of aflatoxin molecule occurs after removal of double bond of the terminal furan ring or opening of lactone ring (*Alberts, 2007).* The structural changes result in loss of fluorescence, and changes in toxicity and mutagenicity. Different enzymes are involved in microbial reduction of $AFLB_1$ presence: F_{420} – dependent reductase in the case of *Actinomicetales* (*Lapalikar et al., 2012*) or laccase in the case of *Peniophora* sp. and *Pleurotus ostreatus (Alberts et al., 2009)*.

It is presumed that two biochemical pathways may be involved in detoxification process of OTA *(Karlovsky, 1999)*. First, OTA can be degraded through the hydrolysis of the amide bond to the non-toxic compounds of the L-*β*-phenylalanine and OTα. Second, a more hypothetical process, involves OTA being transformed via the hydrolysis of the lactone ring, although in this case, the final product of detoxification is an opened lactone form of OTA, which is of similar toxicity to OTA when administered to some laboratory animals *(Abrunhosa et al., 2010)*.

The aim of this study was to determine the role of some fungi, originating from Serbia and not expressing toxigenic properties, in detoxification of $AFLB_1$ and OTA.

Material and Methods

Fungal cultures. Thirty-seven isolates of atoxigenic fungi were selected for investigation of $AFLB_1$ detoxification *in vitro*. The largest number of test fungi (29) belonged to the genus *Aspergillus*: *A. clavatus* Desm. (2), *A. flavus* (5), *A. fumigatus* (7), *A. nidulans* (1), *A. niger* (10) *A. terreus* (1) and 3 *Aspergillus* spp. The remainder of the analyzed fungal species were: *Cephaliophora tropica* Thaxter (1), *Cladosporium* sp. (1), *Eurotium amstelodami* L. Mangin (1), *Fusarium* sp. (1), *Purpureocillium lilacinus* (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson (syn. *Paecilomyces lilacinus* (Thom) Samson) (1), *Penicillium* spp. (2), and *Rhizopus* sp. (1).

Detoxification of OTA was also investigated in laboratory conditions by forty-five isolates of atoxigenic fungi, belonging to genera *Aspergillus* (21), *Fusarium* (3), *Mucor* (3), *Penicillium* (2), *P. lilacinus* (1), *Rhizopus* (13), *Scopulariopsis* (1) and *Trichoderma* (1).

The majority of tested fungi originated from samples of feed and its components that were not mycotoxin-contaminated or were isolated from air in the course of a regular sterility control of premises of laboratory in which microbiological analyses were carried out during 2009-2012 period. The fungal identifications were performed after *Domsh et al. (1980)* and *Luangsa-ard et al. (2011)*. Fungal cultures were maintained on potato dextrose agar (PDA) at 4-6 ºC.

Production and isolation of mycotoxins. Crude micotoxins were produced employing solid substrate fermentations with *A. flavus* isolate GD-2 (leg. prof. dr G. Dimić, Technological Faculty, Novi Sad) for $AFLB_1$ and *A. ochraceus* strain CBS 108.08 for OTA *(Bočarov-Stančić et al., 2009, 2010a)*. Mycotoxins were isolated from the substrate on which the fungi were incubated, and purified according to the thin-layer chromatography (TLC) *(***The Official Gazete of SFRY No. 15, 1987).** ***Mycotoxin extracts were*** evaporated to a dry residue and dissolved

in ethanol to the concentration of 100 μg mL^{-1}($AFLB_1$) and 1000 μg mL^{-1} (OTA), and were stored until used at 4-6ºC.

Experimental procedure. Obtained $AFLB_1$ and OTA were added separately to the modified Vogel's medium *(Vogel, 1956; Bočarov-Stančić et al., 2010b)* to the final concentration of 0.6 μg mL^{-1} for $AFLB_1$ (VAFLA) and 10 μg mL^{-1} for OTA (VAOTA), respectively. The presence of micotoxin residue in the test mediaum was determined after 7 and 14 days of fungal incubation on this medium at 26±1 °C (*Bočarov-Stančić et al., 2010b)*. Discs, cut out of the central part of the fungal colony as well as control discs (with no test fungal culture), were directly placed on TLC plates coated with Kieselgel G (thickness of 2.5 mm) and wetted with 10-20 μL of a chloroform-methanol mixture (2:1, v/v). Several seconds later discs were removed from the TLC plates and chromatography plates were developed together with different volumes of working standard solution of $AFLB_1$ and OTA at concentration of 0.0005 μg μL^{-1} and 0.005 μg μL^{-1}, respectively.

Thin layer chromatography was performed in a saturated tank of t***oulene-ethyl acetate-formic acid*** mixture (5:4:1, v/v/v) in the case of $AFLB_1$ and saturated system of the benzene-acetic acid mixture (9:1, v/v) in the case of OTA, respectively. After the plate development and natural drying in a darkened digester, plates were examined for $AFLB_1$ residues under long wavelength UV rays. OTA was visually detected also under long wavelength UV rays but after TCL plates were sprayed with $NaHCO_3$ solution in ethanol and heated for 10 min at 130°C. All analyses were done in three replicates and average values are presented. Detection limit (LoD) of the applied TLC method amounted to 1.33 μg kg^{-1} for $AFLB_1$ and 2.74 μg kg^{-1} for OTA.

Detoxification rate (%) was calculated by the following formula, where C_o was micotoxin quantity in control disc and C was mycotoxin quantity in disc with fungal colony:

$$Detoxification\ rate = [\frac{C_0 - C}{C_0}]\, x\, 100$$

Results and Discussion

Fungi that detoxificated $AFLB_1$ *in vitro* are presented in Tables 1 and 2. In all cases reduction of $AFLB_1$ concentration was observed after 7 days of fungal incubation on test medium supplemented with this mycotoxin. Prolonged cultivation did not change these results. The best results in detoxification of $AFLB_1$ *in vitro* were achieved by *Cladosporium* sp. and *C. tropica* (Table 1). *Shantha et al. (2000)* found that cultures of *Cladosporium* sp. and *A. terreus* were by far least efficient in $AFLB_1$ biotransformation. Contraty to these results, our isolate of

Cladosporium sp. redused almost all $AFLB_1$ added in the test medium (95.0%). The same result was achieved by C. *tropica.* This mainly coprophilous fungal species, known principally from tropical and subtropical countries *(Domsh et al., 1980)*, was isolated during routine microbiological analysis of sunflower meal from Serbian province of Vojvodina.

Table 1. Detoxification of aflatoxin B_1 by different fungal species (Aspergilli are not included)

No.	*Species*	*Isolate origin*	*Isolate designation*	Detoxification (%)
1.	*Cephaliophora tropica*	Sunflower meal	400/12	95.0
2.	*Cladosporium* sp.	VAFLA	INF-3/12	95.0
3.	*Eurotium amstelodami*	Air	PR-6/11	20.0
4.	*Fusarium* sp.	Sunflower meal	426/12	75.0
5.	*Penicillium* sp.	VAFLA	INF-3/11	90.0
6.	*Penicillium* sp.	VAFLA	INF-4/11	80.0
7.	*Purpureocillium lilacinus*	Air	D-6/11	75.0
8.	*Rhizopus* sp.	Feed mixture	681/12	65.0

Very efficient fungi in $AFLB_1$ detoxification were also *Penicillium* species (80.0-90.0%). *Fusarium* sp. and *P. lilacinus* demonstrated somewhat smaller reduction of $AFLB_1$ concentration in the test medium (75.0%) (Table 1). Results by *Luangsa-ard et al. (2011)* showed that *P. lilacinus* had potential as a biological agent for suppressing of destructive root-knot nematodes. Rather high capability for $AFLB_1$ reduction demonstrated by *Rhizopus* sp. (65.0%) was similar with the results obtained by *El-Shiekh et al. (2007).*

Majority of 29 tested isolates of *Aspergillus* species (1/2 *A. clavatus*, 2/5 *A. flavus*, 5/7 *A. fumigatus*, 1/1 *A. nidulans*, 8/10 *A. niger*, 1/1 *A. terreus* and 3/3 *Aspergillus* spp.) detoxificated $AFLB_1$ during the growth on modified Vogel's agar supplemented with 0.6 μg mL^{-1} of $AFLB_1$.(Table 2).

Table 2. Detoxification of aflatoxin B_1 by *Aspergillus* spp.

No.	*Fungal species*	*Isolate origin*	*Isolate designation*	Detoxification (%)
1.	*A. clavatus*	Air	PR-43/11	85.0
2.	*A. flavus*	Sunflower meal	932-1/12	50.0
3.	*A. flavus*	Feed mixture	523-1/12	85.0
4.	*A. fumigatus*	VAFLA	Inf.-1/11	75.0
5.	*A. fumigatus*	VAFLA	Inf.-2/11	80.0
6.	*A. fumigatus*	Air	PR-45/12	85.0
7.	*A. fumigatus*	Feed mixture	523-2/12	80.0
8.	*A. fumigatus*	Air	PR-43/12	65.0
9.	*A. nidulans*	Sunflower meal	149-A/12	60.0
10.	*A. niger*	Feed mixture	47-2/10	80.0
11.	*A. niger*	Cob	506-2/10	65.0
12.	*A. niger*	Sunflower meal	653-2/12	25.0
13.	*A. niger*	Cob	1292/09	50.0
14.	*A. niger*	Air	D1-1/10	70.0
15.	*A. niger*	Air	D1-2/10	85.0
16.	*A. niger*	Air	D1-3/10	75.0
17.	*A. niger*	Soil	Rb-gr/10	65.0
18.	*A. terreus*	Feed mixture	523-3/12	80.0
19.	*Aspergillus* sp.	Sunflower meal	187/12	85.0
20.	*Aspergillus* sp.	Sunflower meal	653-3/12	50.0
21.	*Aspergillus* sp.	Sunflower meal	932-2/12	25.0

The reduction of $AFLB_1$ in VAFLA varied from 25.0 to 85.0%. The most efficient Aspergilli in $AFLB_1$ detoxification (85.0%) were fungal cultures isolated from the air during routine control of hygiene in microbiological laboratory (PR-43/11 – *A. clavatus*, PR-45/12 – *A. fumigatus* and D1-2/10 – *A. niger*) as well as two isolates from feed (523-1/12 – *A. flavus* and 187/12 – *Aspergillus* sp.). Obtained results that *A. flavus* and *A. niger* can reduce $AFLB_1$ presence are not surprising because other investigators reported simillar results (*Shapira and Paster, 2004; Alberts, 2007)*.

Fungal cultures that detoxificated OTA *in vitro* are presented in Tables 3 and 4. Total of 28.89% fungal isolates had the capability to reduce OTA concentration *in vitro* after 14 days, although few of them detoxificated this mycotoxin after 7 days.

Table 3. Percentage of ochratoxin A detoxifying fungi originating from different sources

No.	*Origin of fungal isolates*	*No. of tested isolates*	*Detoxifying isolates (%)*
1.	Additive (Intraco)	1	100.0
2.	Cereal grains	11	18.9
3.	Oil seeds and products	9	22.2
4.	Feed mixtures	12	25.0
5.	Air	9	33.3
6.	Soil	1	0
7.	Water	1	100.0
	TOTAL	44	27.3

Table 4. *In vitro* mycological detoxification of ochratoxin A by different fungal species

No.	*Fungal species*	*Isolate origin*	*Isolate designation*	*Detoxification (%)*
1.	*Aspergillus fumigatus*	Additive (Intraco)	660/09	30.0
2.	*Aspergillus fumigatus*	Air	D-2/11	20.0
3.	*Aspergillus fumigatus*	Sunflower meal	238/11	70.0
4.	*Aspergillus fumigatus*	Water	V-E/11	10.0
5.	*Aspergillus niger*	Feed mixture	47-2/10	50.0
6.	*Aspergillus niger*	Air	PR-8/10	20.0
7.	*Aspergillus niger*	Barley grain	362/11	50.0
8.	*Aspergillus niger*	Corn grain	365/11	10.0
9.	*Aspergillus niger*	Feed mixture	179/11	10.0
10.	*Purpureocillium lilacinus*	Air	Inf. 2/A/09	90.0
11.	*Rhizopus stolonifer*	Flax seed	1195/09	50.0
12.	*Rhizopus stolonifer*	Feed mixture	186/11	30.0
13.	*Trichoderma* sp.	Air	D-4/11	80.0

We have chosen to test *in vitro* representatives of mentioned fungal genera because there are numerous available literature data that fungi, such as *A. fumigatus* Fres., *A. ochraceus* Wilhelm, and *A. wenti* Wehmer *(Abrunhosa et al., 2010)*, *A. niger* Tiegh., *A. japonicus* Saito *(Bejaoui et al., 2006*), *Rhizopus microsporus* Teigh., *R. homothallicus* Hesseltine & Ellis, *R. oryzae* Went & Prinsen-Geerligs *(Varga et al., 2005)* and others, can detoxify up to 95.0% of the initial OTA amounts.

In our study four out of seven *A. fumigatus* isolates (Table 4) reduced 10.0-70.0% of applied OTA concentration. Isolates of another *Aspergillus* species – *A. niger* (41.7%) detoxified from 10.0 % to 50.0% of OTA (Table 4). All other tested Aspergilli (8) were not capable to reduce presence this mycotoxin.

In the case of *Rhyzopus* spp. only 15.4% of *R. stolonifer* isolates detoxified 30-50% of OTA. The most effective fungal isolates were *Trichoderma* sp. D-4/11 and *P. lilacinus* Inf.2/A/09 (Table 4), that reduced 80.0% and 90.0% of OTA incorporated in modified Vogel's test medium, respectively. These fungal cultures as well as *A. fumigatus* D-2/11 and *A. niger* PR-8/10 (Table 4) were isolated after screening ambient air above working areas in the Department of microbiology of the Bio-Ecological Center in Zenjanin. Other detoxifying mycobiota (Table 3) originated from complete feed mixtures and their components (cereal grains and oilseed products) not contaminated with $AFLB_1$, OTA deoxynivalenol, zearalenone, diacetoxyscirpenol and T-2 toxin.

The amount of OTA (150 μg per Petri dish, eg. 10 μg mL^{-1} in the test medium) used in this experiment for the growth of the analysed mycobiota was significantly higher than the common natural contamination of different substrates with this mycotoxins (about 50 ppb). In similar experiments with OTA detoxification other authors used 0.05 μg mL^{-1} of this mycotoxin *(Böhm et al., 2000)*, which is two hundred-fold lower amount than that used in our experiment. Gained preliminary results point out that some of our fungal isolates have an excellent potential for reduction of OTA presence.

It is interesting to point out that one tested *A. niger* isolate (47-2/10), besides $AFLB_1$ (Table 2) and OTA (Table 4) can detoxify and T-2 toxin *in vitro (Bočarov-Stančić et al., 2010b).* These results indicate that this isolate of *A. niger* is the most promising candidate for environmental remediation as well as feed additive.

Conclusion

Quantity of mycologically detoxificated mycotoxins ranged from 20.0-95.0% ($AFLB_1$) and 10.0-90.0% (OTA), respectively.

Majority of tested fungal species (78.4%) reduced the concentration of $AFLB_1$ *in vitro* while only 28.89% detoxified OTA.

The best results in reduction of the presence of $AFLB_1$ *in vitro* were achieved by *Cladosporium* sp. and *C. tropica* (95.0%) while the most effective fungal isolates in detoxification of OTA were *Trichoderma* sp. isolate D-4/11 (80.0%) and *P. lilacinus* isolate Inf.2/A/09 (90.0%).

A. niger (47-2/10) is the most promising candidate for environmental remediation because it can, detoxificate both $AFLB_1$ and OTA, as well as T-2 toxin, which was previously tested**.**

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Mikološka detoksikacija aflatoksina B_1 i ohratoksina A *in vitro*

A. Bočarov-Stančić, J. Lević, M. Bodroža-Solarov, V. Pantić, N. Salma, J. Vučković

Rezime

U kontrolisanim laboratorijskim uslovima ispitana je sposobnost 37 netoksigenih izolata gljiva u detoksikaciji aflatoksina B_1 ($AFLB_1$) i 45 izolata u detoksikaciji ohratoksina A (OTA). Sirovi ekstrakti mikotoksina su dodati u test podlogu (modifikovana Vogelova podloga) do konačne koncentracije od 0,6 µg mL^{-1} za $AFLB_1$ i 10,0 µg mL^{-1} za OTA. Prisustvo rezidua analiziranog mikotoksina u test podlozi je praćeno nakon 7 i 14 dana inkubacije gljiva na toj podlozi na 26±1°C.

Većina analiziranih Aspergilla (1/2 *A. clavatus*, 2/5 *A. flavus*, 5/7 *A. fumigatus*, 1/1 *A. nidulans*, 8/10 *A. niger*, 1/1 *A. terreus* i 2/3 *Aspergillus* spp.) je smanjila koncentraciju $AFLB_1$ od 20,0 do 85,0% već posle 7 dana inkubacije. Najveću sposobnost smanjenja prisustva $AFLB_1$ *in vitro* (95,0%) su pokazale vrste *Cladosporium* sp. i *Cephaliophora tropica.*

Ukupno 28,89% izolata gljiva imalo je potencijala da detoksifikuju OTA *in vitro* nakon 14 dana, a u nekim slučajevima već i nakon 7 dana. Izolati vrsta *A. fumigatus* (57,1%) su za 10,0-70,0% smanjili koncentraciju OTA, *A. niger* (41,7%) za 10,0%-50,0% i *R. stolonifer* (15,4%) za 30,0-50,0%. Izolati vrsta

Trichoderma sp. i *P. lilacinus* su bili najefikasniji u smanjenju koncentracije ovog mikotoksina. Ove vrste su za 80,0-90,0% smanjile koncentraciju OTA, koji je bio dodat u test podlogu.

Dobijeni rezultati pokazuju da jedan od testiranih izolata vrste *A. niger* (47-2/10) najviše obećava kao kandidat za sanaciju životne sredine i kao aditiv hrani za životinje, jer istovremeno detoksifikuje $AFLB_1$ i OTA.

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EFFECT OF DIETARY PROBIOTIC ON EGG QUALITY

M. Ignatova, V. Sredkova, E. Petkov, M. Todorova

Institute of Animal Science, Kostinbrod, Bulgaria
Corresponding author: maia_ignatova@abv.bg
Original scientific paper

Abstract: The aim of this experiment was to investigate the effect of supplemental probiotic in laying hens diet on morphological parameters, egg quality and yolk cholesterol concentration. The probiotic containing Enterococcus faecium ($5x10^{12}$ CFU) was added to the diet at quantity of 200 g/t. In this study, were used two egg type lines birds (L and HL) from gene pool flock of the experimental poultry farm of Institute of Animal Science – Kostinbrod. The hens of each egg type line were randomly allocated to two groups – control and experimental. The hens of the control groups received standard compound feed without added supplement. The hens of the experimental groups were fed with standard compound feed supplemented with probiotic. Egg weight and egg albumen were significantly higher for the experimental group HL ($P<0.01$; $P<0.001$), while for the experimental group L these parameters were not affected by treatment. The Haugh unit was significantly improved for both experimental groups ($P<0.01$) compared to the controls, but egg shell qualities were not affected. The results showed that in groups received probiotic the intensity of yolk color was increased. Yolk cholesterol concentration in eggs was reduced for the experimental groups compared to the control groups.

Key words: laying hens, probiotic, egg quality, yolk cholesterol.

Introduction

Probiotics usually defined as live microorganisms which, when administered in adequate amounts, confer a health benefits on the host *(Casey et al., 2007)*. The most commonly used as probiotics microbial species are Lactobacillus spp., Biffidobacterium spp., Enterococcus spp., Streptococcus spp. and Bacillus spp. strains *(Yörük et al., 2004; Xu et al., 2006; Casey et al., 2007)*. Probiotics act as stabilizers of the intestinal flora *(Gallazzi et al., 2008)*. Mechanism of action of probiotics include alteration in intestinal flora, enhancement of growth of nonpathogenic facultative anaerobic and gram positive bacteria forming lactic acid and hydrogen peroxide, suppression of growth of

intestinal pathogens, and enhancement of digestion and utilization of nutrients *(Yörük et al., 2004).*

The research studies have shown that inclusion of probiotics in laying hens diet improved egg production, egg size and egg quality *(Yörük et al., 2004; Bageridizaj et al., 2006; Xu et al., 2006; Yousefi and Karkoodi, 2007; Kalavathy et al., 2009)*. Improved gut health enhance feed efficiency *(Bageridizaj et al., 2006; Gallazzi et al., 2008)* probiotics also decreased yolk cholesterol concentration *(Mahdavi et al., 2005; Xu et al., 2006; Kalavathy et al., 2009)*.

The purpose of this experiment was to study the effect of probiotics inclusion in diet of two type laying hens on morphological parameters, egg quality and yolk cholesterol concentration.

Material and methods

The experiment was conducted with 400 laying hens from two egg type lines ("L" and "HL") from gene pool flock of experimental poultry farm of Institute of animal science – Kostinbrod. Each type line hens were divided into two groups – control (100 birds) and experimental (100 birds). Control groups were fed with standard compound feed. Experimental groups received compound feed supplemented with probiotic contained Enterococcus faecium ($5x10^{12}$ CFU) at quantity of 200g/t feed.

At the end of the experiment fifteen eggs from each group were randomly selected and were used to explore morphological parameters, egg quality and yolk cholesterol concentration.

The explored parameters were:

- Egg weight;
- Egg shape index;
- Egg shell thickness;
- Egg shell weight;
- Albumen index;
- Albumen weight;
- Yolk index;
- Yolk weight;
- Haugh units;
- Roche units;
- Yolk cholesterol concentration.

The research data analyses were made by the usual variation statistics methods and Student`s t – test was used to compared means.

Results and discussion

The results for morphological parameters and egg quality of this experiment are shown in table 1. Egg weight were significant higher for the "HL" experimental group (P<0.01) compared to the "HL" control group. These results are in agreement with the results of other studies *(Na et al., 2003; Kalavathy et al., 2009)*. There were no differences in this parameter for the "L" groups. Similar results were observed in other studies *(Yörük et al., 2004; Yousefi and Karkoodi, 2007)*.

Table 1: Morphological and quality parameters of eggs (x□ ± SD).

Parameters	Groups "HL" type		Groups "L" type	
	Control	Experimental	Control	Experimental
Egg Weight, g	52.13±2.29	55.80±2.88**	53.87±3.81	53.20±2.21
Shape index of egg	77.18±3.03	76.77±2.51*	76.48±2.52	78.55±3.24*
Albumen index	122.16±10.94	130.33±13.53*	122.22±13.63	136.63±13.97**
Albumen weight, g	33.21±1.90	36.64±2.69***	34.23±3.28	34.51±1.95
Yolk index	47.02±2.08	46.79±2.02	46.14±1.44	47.64±2.34*
Yolk Weight, g	13.73±0.81	13.85±1.07	14.22±1.07	13.52±1.08*
Egg shell weight, g	5.19±0.36	5.31±0.35	5.42±0.64	5.44±0.57
Egg shell thickness	0.39±0.02	0.38±0.03	0.39±0.03	0.38±0.03
Haugh unit	93.0±2.53	96.27±3.28**	93.93±3.47	97.07±3.30**
Roche unit	7.6±0.74	8.33±0.90*	7.47±0.83	8.20±0.77*

*P<0.05; **P<0.01; ***P<0.001.

Shape index of eggs were significantly improved for experimental groups by probiotic inclusion (P<0.05).

Albumen index was significant improved for the experimental groups. This is in contrast with the research of *Bageridizaj et al., 2006*, who did not observe any differences in this parameter between the groups.

Like egg weight, egg albumen weight is significantly higher for the "HL" experimental group compared to "HL" control group (P<0.001), but for the "L" groups there were no differences.

We observed no differences in yolk index and weight and egg shell thickness and weight between the groups.

Haugh units and yolk color were significantly improved in both experimental groups (P<0.01). These results are in agreement with *Xu et al., 2006*, but are in contrast with the results of *Bageridizaj et al., 2006*, who observed no differences in these parameters.

Yolk cholesterol concentration was lowered for the both experimental groups (table 2). Our results are in agreement with other research studies *(Mahdavi et al., 2005; Xu et al., 2006; Yousefi and Karkoodi, 2007; Kalavathy et al., 2009)*.

Table 2: Yolk cholesterol content (x□ ± SD), mg/100g.

Groups "HL" type		%	Groups "L" type		%
Control	Experimental		Control	Experimental	
1511.55±154.93	1403.83±101.18	107.7	1514.62±40.55	1412.28±105.15*	107.2

*P<0.05

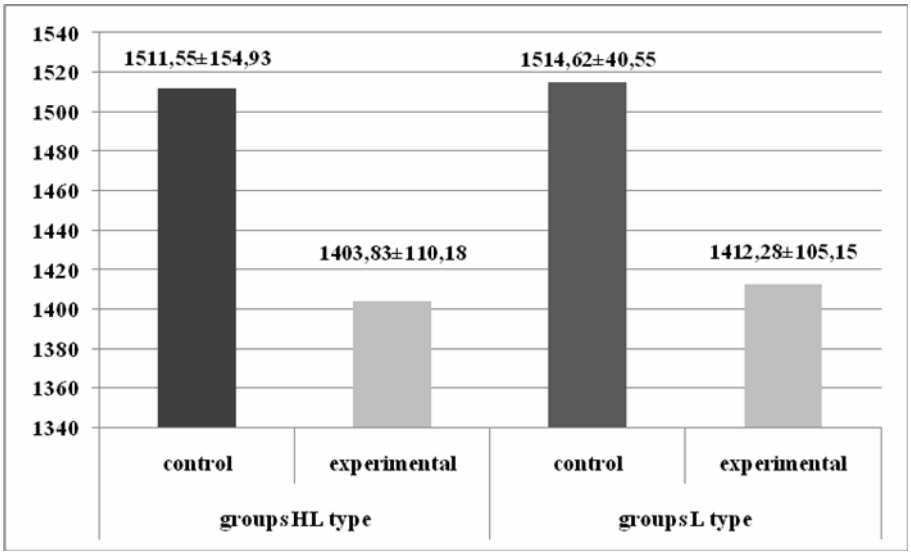

Figure 1: Yolk cholesterol content, mg/100g.

Conclusion

Our study shown that probiotic supplementation in laying hens diets have positive effect on egg shape, albumen index, Haugh units, yolk color and yolk cholesterol content. Egg weight and albumen weight were improved for "HL"

experimental group by probiotic addition, but for the “L” groups there were no differences.

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Efekat korišćenja probiotika u ishrani na kvalitet jaja

M. Ignatova, V. Sredkova, E. Petkov, M. Todorova

Rezime

Mnoge studije su pokazale da probiotici u ishrani nosilja imaju blagotvorno dejstvo na neke morfološke osobine i parametre kvaliteta jaja.

Ovaj eksperiment je sproveden kako bi se ocenio efekat suplementacije probiotika u ishrani nosilja na morfološke parametre, kvalitet jaja i sadržaj holesterola u žumancu.

Eksperiment je izveden na 400 kokoši nosilja dve linije tipa jaja (tip "HL" i "L") iz jata genofonda na eksperimentalnoj živinarskoj farmi Instituta za stočarstvo - Kostinbrod. Ptice su dodeljivanje jednoj od četiri grupe - "HL" kontrolna i eksperimentalna grupa, kao i "L" kontrolna i eksperimentalna grupa. Svaka grupa sadržala je 100 nosilja. Kontrolne grupe su hranjene smešom bez dodatka. Eksperimentalne grupe su hranjene smešom dopunjenom sa probiotikom *Enterococcus faecium* ($5x10^{12}$ CFU) u količini od 200g/t hrane.

Kontrolisani parametri bili su: težina jajeta, indeks oblika jajeta, debljine ljuske jajeta; težina ljuske jajeta, indeks belanca, težina belanca, indeks žumanca, težina žumanca, Haugove jedinice, Roche jedinice, holesterol u žumancetu.

Rezultati ove studije pokazali su da je suplementacija probiotika značajno popravila Haugove jedinice, boju žumanca, indeks belanca i indeks oblika u obe eksperimentalne grupe. Težina jaja i belanca je povećana samo kod "HL" eksperimentalne grupe. Za "L" ogledne grupe nije bilo razlike u ovim parametrima. Sadržaj holesterola u žumancu eksperimentalnih grupa bio je manji u odnosu na kontrolne grupe. Nisu utvrđene razlike između grupa u drugim istraživanih parametarima: debljina ljuske jajeta; masa ljuske jajeta, indeks žumanca, težina žumanca, između grupa.

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PLUMAGE CONDITION AND FOOT PAD LESIONS OF MEDIUM GROWING BROILERS REARED EXTENSIVELY IN THE POULTRY HOUSE AND IN TRADITIONAL FREE RANGE

M. Đukić Stojčić, N. Milošević, L. Perić, S. Bjedov

Universitiy of Novi Sad, Faculty of Agriculture, 21000, Novi Sad, Republic of Serbia;
Corresponding author: djukic@polj.uns.ac.rs
Original scientific paper

Abstract: Plumage condition and foot pad lesions were measured on Red Bro chickens reared in two different production systems: extensively in the poultry house and traditional free range system. The experiment was carried out on 200 chickens divided into two groupswith four subgroups (25 chickens each). Plumage condition and severity of foot pad lesions were determined at the age of 63 days. Results of the trail showed that Red Bro medium-growing broilers reared both extensively in the poultry house as well as in traditional free range system had good feather condition. It was found that broilers in both systems had problems with foot pad dermatitis, but chickens reared in free range system had a lower incidence of foot pad lesions.

Kay words: broilers, housing system, plumage condition, foot pad lesion

Introduction

Many authors have investigated the welfare of the poultry in different housing systems, with different lighting programmes, litter types, hybrid types or bird densities. In organic and free range chicken production the European regulation suggests the use of indigenous breeds with slow-growing profile, high livability, resistance to diseases, and adaptability to outdoor local conditions. However, in practice mostly fast growing chickens are used due to their higher efficiency although they were selected for indoor rearing condition (*Meluzzi et al., 2009*).

Some of the main factors that affect welfare of chickens are housing system and stocking density. Criteria used to characterize the health and welfare of broilers amongst others are feather condition and incidence of foot pad lesion.

The foot pad dermatitis is the most predominant problem at high-stocking densities and in fast-growing strains (*Dawkins et al., 2004*). An important factor

influencing the development of foot pad lesions is the housing system (*Keutgen et al., 1999; Weitzenbürger et al., 2005*). The defects of plumage condition are also seen to be affected by housing system (*Rönchen et al., 2007*). *Kjaer et al. (2006)* found no foot pad dermatitis lesions in chickens from the slow-growing strain. In the fast-growing strain, the first signs of foot pad dermatitis were seen in week 2. The incidence of lesions increased thereafter. Also, *Meluzzi et al. (2009)* found that incidence of foot pad lesion was higher in fast-growing broilers in comparison with medium-and slow-growing. Fast-growing broilers also had worse plumage condition. *Ferranate et al. (2006)* confirmed that the higher plumage damage and foot-pad lesions scores were in broilers reared on a straw litter with higher density. On the other hand *Škrbić et al. (2009)* concluded that reduction of stocking density from 16 to 13, or 10 birds/m2 in intensive broiler production doesn't have significant effect on broiler welfare parameters (walking ability, incidence and degree of hock burns, foot pad lesions and feathering).

The objective of the present study was to evaluate a plumage conditions and incidence of foot pad lesions of medium-growing broilers, reared in the poultry house or in traditional free range system.

Material and Methods

The experiment was carried out on Red Bro meat-type strains, medium-growing broilers. Two hundred chickens were divided into two groups and reared in two different production systems: extensively in the poultry houseor traditional free range system. Both groups were divided into four subgroups with 25 chickens each. Each subgroup had a stocking density of 11 birds/m^2. Until the age of 28 days both groups were reared extensively in the poultry house in a pen floors covered with straw. After 4 weeks 100 broilers were transferred and reared in free range system. In free range system a standard deep litter house was combined with an outdoor range (3 m² of a pasture area were provided per broiler). In the pasture area small shelters for protection from predators and from the sun were placed.

Birds of both groups were fed *ad libitum* with three consecutive feeds. Broiler starter diet was used in the period 1- 4 weeks (21,21 % crude protein, and 12.09 MJ/kg ME), grower mixture in the period 5-7 weeks of age (19,00 % crude protein, 12.80 MJ/kg ME) and finisher was used in the period 8-9 weeks (18,50 % crude protein, 13.57 MJ/kg ME). Basic environmental parameters as well as the lighting program were in accordance to the technological demands of used hybrids.

In 9^{th} week of age the feather condition was investigated on 52 broilers per group. The degree of feather damage was quantified in the six regions: head, neck, breast, wings, back, and tail using a scale from 1 (poorly feathered) to 4 (fully feathered), according to the methods described by *Tauson et al. (1984)* and *Keppler*

et al. (2001), "4" being fully feathered without disorder, and "1" being significantly affected by the disorder. The six regions for plumage condition were summarized and indicated a total score ranging from 6 to 24 points.
The foot pad lesions were evaluated at the end of the 9th week on 52 birds from each treatment and were calculated. Lesions were scored for both the left and right foot and classified according to a scale from 0 to 3, where zero represents no lesion and three the highest severity of damage (*Martrenchar et al., 2007*).

Data were analyzed by ANOVA and means were separated by Duncan's post hoc test using StatSoft computer package (*Statistica 11, 2012*).

Results and discussion

Results of investigation of the total plumage condition and plumage scoring of the different body regions are presented in table 1. Based on average values of the evaluation of the total plumage condition, good feather condition in both chicken groups was established. Small damage in both groups was found at the tail. Housing system significantly affects the plumage status on breast and the wings. The plumage scoring of breast region was better in broilers kept in free range. Broilers reared in the house showed more damage on breast feathers, probably as more time lying on the pen floors. The broilers reared in free range system had on the wings more feather damage compared to broilers kept indoors, who have completely undamaged feathers on the wings. Broilers reared in free range had access to the pasture, showed more activity, more dust and sun bathing and more scratching. All mentioned factors are probably the cause why their feathers on the wings were more damaged compared to broilers in a poultry house. Our results are consistent with studies of *Mahboub (2004*) who observed that birds in free range spent more time in dust bathing, foraging and scratching. The damage on the primary wing feathers and tail may be also attributed to abrasion at the walls of the passages between inside and outside areas.

Table 1. Average value with their standard errors for the total plumage condition and plumage scoring of the different body regions of broilers at 9 weeks of age

Trait	Housing system	
	Free range	Poultry house
Head	4.00 ± 0.00	4.00 ± 0.00
Neck	4.00 ± 0.00	4.00 ± 0.00
Back	4.00 ± 0.00	3.95 ± 0.44
Breast	4.00 ± 0.00[a]	3.85 ± 0.36[b]
Wings	3.62 ± 0.53[b]	4.00 ± 0.00[a]
Tail	3.72 ± 0.50	3.74 ± 0.53
Total plumage condition	23.34 ± 0.21	23.09 ± 0.44

[a-b] Values within column with no common superscript are significantly different (P<0.05)

The foot pad lesions were evaluated at the end of the 9th week and are presented in table 2. We found that incidence of foot pad lesion was higher in broilers kept in poultry house in comparison with broilers from free range. Superficial lesions of the foot pads were found in both housing systems (degree 1). Deep epithelial lesions of foot pad (degree 3) were found only in broilers kept in poultry house (3,85%). The frequency of degree 2 also was higher in broilers from poultry house.

Table 2. Impact of housing system on the incidence of foot pad lesions of broilers at 9 weeks of age

Housing system	Average Score	n	Frequency, %			
			0	1	2	3
Free range	0.48	52	55.77	40.38	3.85	0.00
Poultry house	0.71	52	48.08	36.54	11.54	3.85

Kjaer et al. (2006) and *Meluzzi et al. (2009),* found no foot pad dermatitis lesions in chickens of the slow-growing strain. They reported a higher incidence of foot pad lesion in fast-growing broilers in comparison with medium- and slow-growing.

The Red Bro medium-growing broilers reared in the poultry house had lower foot pad lesions compared to fast-growing broilers reared in a lower stocking density (*Dawkins et al., 2004*;. *Ferrante et al. 2006)*. On the other hand *Škrbić et al. (2009)* reported a very low incidence of foot pad lesions in fast-growing broilers reared in poultry house regardless of stocking density.

Conclusion

Generally it can be concluded that Red Bro medium-growing broilers reared in two different housings systems (extensively in the poultry house and traditional free range) had a good feather condition. It was found that broilers in both systems had problems with foot pad dermatitis, but chickens reared in free range system had a lower incidence of foot pad lesions.

Acknowledgments

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Stanje perja i lezija na nožnim jastučićima pilića srednjeg porasta uzgajanih ekstenzivno u živinarniku i tradicionalnom ispustu

M. Đukić Stojčić, N. Milošević, L. Perić, S. Bjedov

Rezime

Stanje perja i lezija na nožnim jastučićima je određivano na Red Bro pilićima uzgajanim u dva različita sistema držanja: ekstenzivno u živinarniku i tradicionalnom ispustu. Ogled je izveden na 200 pilića podeljenih u dve grupe sa po četiri podgrupe (25 pilića u svakoj). Stanje perja i lezija na nožnim jastučićima utvrđivani su u uzrastu od 63 dana. Rezultati istraživanja su pokazali da su Red Bro srednje rastući brojleri gajeni ekstenzivno u živinarniku, kao i u tradicionalnom ispustu, imali odlično stanje perja. Utvrđeno je da su u oba sistema držanja pilići imali problema sa dermatitisom na nožnim jastučićima, ali da su pilići gajeni u sistemu tradicionalnog ispusta, imali manju učestalost lezija na nožnim jastučićima.

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LIPID PEROXIDATION AND MOTILITY OF DRAKE SPERMATOZOA AFTER 24 HOURS OF STORAGE

R. Stefanov[1], M. Chervenkov[1], E. Kistanova[1], D. Kacheva[1], B. Georgiev[1], P. Taushanova[1], G. Nenkova[2], A. Aleksandrova [2]

[1]Institute of Biology and Immunology of Reproduction, BAS, Tzarigradsko shose 73, 1113, Sofia, Bulgaria
[2]Institute of Neurobiology, BAS, Acad. G. Bonchev St. 23, 1113, Sofia, Bulgaria
Corresponding author: stefanovrossen@gmail.com
Original scientific paper

Abstract: The data on the basic functional characteristics of spermatozoa from waterfowls is very scanty in the scientific literature. It is well known that in main agricultural animals the storage of semen leads to increased levels of lipid peroxidation and changes in sperm quality. In this way is interesting to study the relationship between the lipid peroxidation levels and the changes in biological properties of the sperm in waterfowls. The aim of present work was a determination whether the content of malondialdehyde (MDA), an indicator of the degree of lipid peroxidation, is related to sperm motility in Muscovy drake. The ejaculates were collected by using of artificial vagina. Motility of the spermatozoa was defined by Sperm Class Analyzer (Microoptic, Spain) and the amount of MDA was measured by Specol 11 (Carl Zeiss Jena). Spermatozoa with progressive motility on the day of obtaining were 18.28 ± 0.043% (average for all ejaculates). After 24 hours of storage, this percentage decreased to 5.55 ± 0.025%. There was increase of the average values of MDA content in the ejaculates from 0.86 ± 0.211 nmol/20x10^6cells on the day of collection to 1.323 ± 0.431 nmol/20x10^6cells after 24 hours of storage. Correlation between MDA content and spermatozoa with non-progressive motility at 24 hour of storage (y=-.5842+0.0288*x). It was found increased MDA content after storage of the ejaculates for 24 hours. Also, there was decreased sperm motility, more strongly expressed in the spermatozoa with progressive than those with not progressive movement.

Key words: Muscovy drake, spermatozoa, motility, lipid peroxidation

Introduction

The increased level of lipid peroxidation (LPO) in the semen is one of the reasons for its quality decline. Such data have been obtained in various farm animals as boars *(Kumaresan et al., 2009)*, rams *(Jones and Mann, 1976, 1977a,*

1977b), bulls *(Jones and Mann, 1977a; Slaweta et al., 1988)*.Studies of the relationship between oxidative stress and sperm parameters in poultry indicate difference between gallinaceous birds and waterfowls.Particularly interesting results were obtained from *Partyka et al. (2012)*, who established a higher levels of lipid peroxidation and more strongly expressed oxidative stress in semen of ganders than in partridge roosters. The higher levels of LPO in ganders have been also accompanied with higher percent of spermatozoa with damaged plasma membrane, while in partridge roosters along with the lower levels of LPO there was also lower percent of spermatozoa with impaired membranes. Meanwhile, according to some authors lipid manipulation of chicken semen by dietary means affects their fertility *(Cerolini et al., 2003*)

To the data with respect to Muscovy drake, have been conducted researches on some biochemical parameters of semen *(Atanasov et al., 2007)*, and changes in the speed parameters of sperms, after short-term storage at 4ºC *(Gerzilov et al., 2011)*. The ultrastructural changes in spermatozoa from Muscovy drakes after cryopreservation were also studied *(Kazachka et al., 2008).*

The aim of present work was a determination whether the content of malondialdehyde (MDA), an indicator of the degree of lipid peroxidation, is related to sperm motility in Muscovy drake.

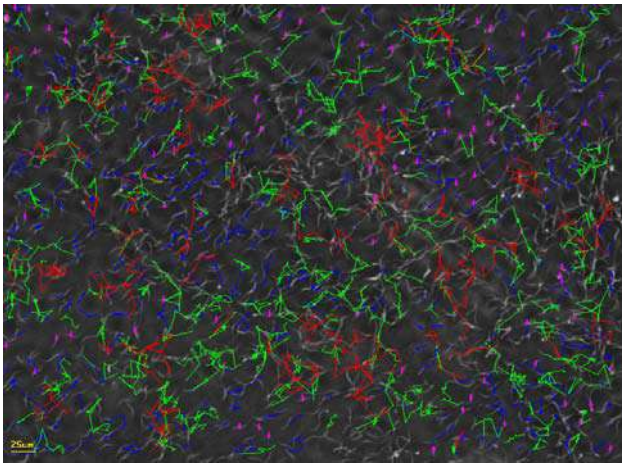
Material and Methods

For the experiment were used clinically healthy, sexually mature drakes from the species Carina moschata.

The semen collection was performed by artificial vagina, according to the method, previously described by *Gerzilov (2000)*

Assesment of motility and concentration of spermatozoa. After obtaining, each ejaculate was diluted 1:5 with semen extender IMV-buffer (IMV - Technologies - France). From each diluted ejaculates were taken 10 μL sample and placed in disposable cameras LEJA 20 for subsequent examination on a computer spermanalyzer SCA (Microptic, Spain). The measurements were made on the day of obtaining and after 24 hours of storage at 0-4 °C.

Visualization of analyzed (by SCA) ejaculate from Muscovy drake is presented in Pic. 1



Picture 1. Image from analyzed ejaculate from Muscovy drake (spermanalyzer SCA (Microptic, Spain).
Rapid progressive motility – red; Non – progressive motility – blue; Slow progressive motility – green; Immotile - pink

Determination of MDA content in semen. From all single ejaculates were taken samples for determination of MDA amount. Each sample was additionally diluted to a concentration of 20 Insert, Symbol $\times 10^6$. Subsequently the samples was frozen - thawed 3 times and centrifuged (3000× g, 5 min, 4 ºC). After centrifugation the supernatant was used for the assay.

Lipid peroxidation was determined by the amount of thiobarbituric acid reactive substances (TBARs), formed in sperm after addition of 0.6 ml of 2,8 % trichloroacetic acid + 5n HCI + 2% thiobarbituric acid in 50 mM NaOH (2:1:2 v/v) to 1 ml of sperm suspension. The samples were heated at 100 ºC for 15 min till color development. After cooling, the suspension was centrifuged at 1,000 × g for 10 minutes. The supernatant was separated, and the absorbance was measured at 532 nm against appropriate blank. The 600 nm absorbance was considered to be a nonspecific base-line and was subtracted from A_{532}. The assay was conducted in triplicate using a double beam spectrophotometer Specol 11 (Carl Zeiss Jena). The values were expressed in nmoles MDA/ml, using a molar extinction coefficient of $1{,}56 \times 10^5$ M^{-1} cm^{-1}.

Statistical analysis. Data were processed by computer statistical package STATISTICA (Ver.6.0 of the Stat Soft Inc.). The significance of differences were determined by t - test.

Results and Discussion

The data, presented in Table 1 show, that spermatozoa with progressive motility on the day of obtaining were 18.28 ± 0.043% (average for all ejaculates). After 24 hours of storage, this percentage decreased to 5.55 ± 0.025%. The same trend was observed in spermatozoa with non-progressive motility - 75.22 ± 0.109% on the day of obtaining and 66.12 ± 0.090 after 24 hours of storage.

Table 1. Motility of drake spermatozoa at the time of ejaculates obtaining and after 24 hours of storage (n = 6)

Drake ID	Motility of spermatozoa in ejaculates, immediately after obtaining (%)			Motility of spermatozoa in ejaculates, following 24 h. of storage (%)			Concentration millions per ml
	Static	Non-progressive motile	Progressive motile	Static	Non-progressive motile	Progressive motile	
1	0.1	77.0	22.9	38.0	59.5	2.5	897.1
2	4.2	80.9	14.9	19.4	72.0	8.6	487.5
3	6.5	81.6	11.9	35.2	56.7	8.1	815.0
4	0.5	77.7	21.7	35.2	61.8	3.0	577.5
5	25.9	53.2	20.9	29.1	65.9	5.0	465.6
6	1.6	80.9	17.4	13.0	80.8	6.1	739.7
Average	6.47± 9,8	75.22± 10,9	18.28± 4, 3	28.32± 10,0[a]	66.12± 8,9	5.55± 2, 5[a]	663.7± 179.368

[a] - P< 0,01 compared to the 0 hour

Data are $\bar{x}$ ± SD

In Table 2 are presented the changes in the quantity of MDA in the ejaculates before and after storage. There was increase of the average values of MDA content in the ejaculates from 0.86 ± 0.211 nmol/20x10^6cells on the day of collection to 1.323 ± 0.431 nmol/20x10^6cells after 24 hours of storage. The changes n the MDA contents at 24 hour have positive relationship with number of spermatozoa with non-progressive motility (Fig.1).

Table 2. The changes of the MDA content in drake ejaculates during the 24 hours of storage (n=6)

Drake ID	MDA 0 hour (nmol/20x10^{6}cells)	MDA 24 hour (nmol/20x10^{6}cells)
1	1.262	1.688
2	0.882	1.334
3	0.728	0.923
4	0.79	1.159
5	0.667	0.872
6	0.831	1.96
Average	0.86 ±0.211	1.323±0.431^b

Data are $\bar{x}$ ± SD

b - P < 0,05 compared to the 0 hour

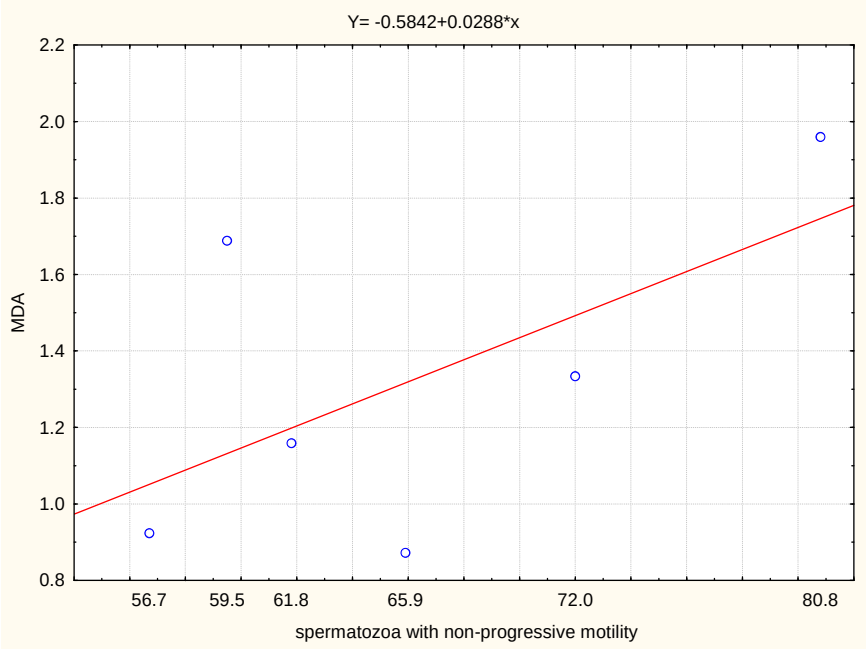


Figure 1. Correlation between MDA content and spermatozoa with non-progressive motility at 24 hour of storage

Correlation between MDA content and spermatozoa with non-progressive motility at 24 hour of storage (y=-.5842+0.0288*x)

Conclusion

It was found significant decreasing of the total motility of the spermatozoa in the Muscovy drakes ejaculates, during storage at 0-4 ºC for 24 hours. That was more strongly expressed in the spermatozoa with progressive - motility than those with non-progressive motility (P=0,006).

At the same time, the amount of MDA, an indicator of the lipid peroxidation, increased almost two fold. Decreasing the spermatozoa motility in Muscovy drake ejaculates during the 24 hours of storage corresponds with the rising of lipid peroxidation levels. Particularly, the positive correlation was established between spermatozoa with non- progressive motility and MDA content in ejaculates at 24 hour (r=0,60)

In accordance with the obtained data, we assume that storage of Muscovy drake semen at 0-4 °C, for more than 24 hours is ineffective.

Lipidna peroksidacija i pokretljivost spermatozoida plovki posle 24 sata skladištenja

R. Stefanov, M. Chervenkov, E. Kistanova, D. Kacheva, B. Georgiev, P. Taushanova, G. Nenkova, A. Aleksandrova

Rezime

Podaci o osnovnim funkcionalnim karakteristikama spermatozoida plovki su veoma retki u naučnoj literaturi. Poznato je da kod glavnih vrsta poljoprivrednih životinja skladištenje semena dovodi do porasta nivoa lipidne peroksidacije i promena u kvalitetu sperme. Na ovaj način je interesantno da se ispita povezanost između nivoa lipidne peroksidacije i promena u biološkim osobinama sperme plovki. Cilj ovog rada bio je da se utvri da li je sadržaj malondialdehida (MDA), kao jednog od pokazatelja stepena lipidne peroksidacije, u vezi sa pokretljivošću spermatozoida mošusne patke. Ejakulati su prikupljeni pomoću veštačke vagine. Pokretljivost spermatozoida definisana je korišćenjem Sperm Class Analyzer (Microoptic, Spain) i iznos MDA je meren pomoću Specol 11 (Carl Zeiss Jena). Procenat spermatozoida sa progresivnim motilitetom na dan preuzimanja bio je 18,28 ± 0,043% (prosek za sve ejakulate). Nakon 24 sata od skladištenja, ovaj procenat je opao na 5,55 ± 0.025%. Utvrđeno je povećanje prosečnih vrednosti sadržaja MDA u ejakulatima od 0.86 ± 0.211 nmol/20x10^6 ćelija na dan sakupljanja sperme, do1.323 ± 0.431 nmol/20x10^6 ćelija posle 24 sata skladištenja. Korelacija

između MDA i sadržaja spermatozoida sa ne-progresivnom pokretljivošću nakon 24 sata skladištenja (*y*=-.5842+0.0288*x). Utvrđeno je povećanje sadržaja MDA nakon skladištenja ejakulata u trajanju od 24 sata. Takođe, smanjena pokretljivost spermatozoida, jače je izražena kod spermatozoidima sa progresivnim od onih sa ne-progresivnim kretanjem.

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EFFECT OF PRESTORAGE INCUBATION ON HATCHABILITY OF LONG-TERM STORED BROILER BREEDER EGGS

L. Perić[1], D. Žikić[1], M. Đukić Stojčić[1]

[1]Univerzitet u Novom Sadu, Poljoprivredni fakultet, Trg Dositeja Obradovića 8, 21000 Novi Sad, Republic of Serbia
Corresponding author: lidija.peric@stocarstvo.edu.rs
Original scientific paper

Abstract: The trial is based on a concept that short incubation of eggs prior to prolonged storage (PRESI) will have positive effect on a survival of embryos. A total of 1120 broiler breeder eggs were collected from a commercial broiler breeders farm and divided into four groups representing four pre-storage incubation periods (PRESI): 0 h (control), 3 h of PRESI, 6 h of PRESI and 9 h of PRESI. After treatments eggs were stored for 10 days at temperature of 16°C and relative humidity of 65-75%. Upon completition of storage period, all eggs were incubated and following results were achieved: average hatchability of control group was 85.21%, for 3 h PRESI 87.69 %, for 6 h PRESI 83.46% and for 9 h PRESI 79.61%. From the results of the trial, it can be stated that PRESI treatment in duration 3 hours has positive effect on hatchability of eggs stored for 10 days, while PRESI treatment for 9 h prior to storage has detrimental effect on embryo development and hatching.

Key words: prestorage incubation, eggs, hatchability

Introduction

Optimal period for storage of setting eggs is from 3 to 7 days. Sometimes, producers are forced to store setting eggs for a longer period of time for different reasons, and that has a detrimental effect on hatchability. Long term storage of hatching eggs has been thoroughly investigated and it is well known that extended periods of egg storage depress hatchability, prolong incubation time, and can adversely affect chick quality *(Meijerhof, 1992; Fasenko et al., 2001a; Ates et al., 2004)*. Many researchers established that storage of eggs longer than a week increases embryonic abnormalities and mortality (*Van der Ven, 2004; Fasenko, 2007*). According to *Yasin et al. (2008)* every extra day of storage until day 7 reduces hatchability for 0.2% and from day 7 to 14 for 0.5%.

Some previously published studies have associated hatchability of chickens with the stage of embryonic development at the time the egg is stored *(Fasenko et al., 2001a; Petek and Dikmen, 2004, Jotanović et al., 2010)*. The hypothesis is that if the embryo is at the more advanced stage of embryonic development it might have a better chance to survive a long term storage period. *Fasenko et al.(2001b)* determined that when turkey eggs are exposed to prestorage incubation (PRESI) for a short time and then stored for 14 days, embryos that have completed hypoblast formation (12 h of PRESI) have a survival advantage over embryos that are undergoing, or have just completed, area pellucida formation (0 h of PRESI).

The aim of this trial was to establish the effect of different duration of prestorage incubation on hatchability and embryonic mortality.

Materials and Methods

A total of 1120 broiler breeder eggs were collected from a commercial broiler breeding farm. Brioler breeder strain was ROSS 308 at the age of 30 weeks. Eggs were divided into four groups and exposed to four pre-storage incubation periods (PRESI): 0 h (no prestorage heating - control), 3 h of PRESI, 6 h of PRESI and 9 h of PRESI. After treatments eggs were stored for 10 days at temperature of 16°C and relative humidity of 65-75%. Upon completition of storage period, all eggs were set in commercial incubator. Control candling was at 7th day of incubation. Unfertilized eggs and early embrionic mortality was determined. At the end of incubation, time and cause of embryonic mortality were determined for all unhatched eggs.

Data were analysed by ANOVA using the GLM procedure and means were separated by Duncans *post* hoc test using StatSoft computer package (STATISTICA 10, 2011). All data presented in percentage were transformed using arcsin transformation prior to testing.

Results and Discussion

The effects of PRESI treatments on incubation results are presented in Table 1.

Table1. The effect of prestorage incubation on incubation results

Parameter	Duration of prestorage incubation			
	0	3h	6h	9h
Number of set eggs (n)	270	260	260	260
Number of fertile eggs (n)	253	249	242	243
Fresh egg weight (g)	57.81	57.64	57.80	57.94
Egg weight loss during storage (%)	1.06	1.08	1.12	1.20
Hatchability of total eggs (%)	85.21^{AB}	87.69^{A}	83.46^{AB}	79.61^{B}
Hatchability of fertile eggs (%)	90.92^{ab}	91.62^{a}	89.67^{ab}	85.18^{b}

a-b Values within the rows with no common superscript are significantly different (P<0.05)
A-B Values within the rows with no common superscript are significantly different (P<0.01)

There was no significant effect of duration of PRESI treatments on the egg weight loss during storage. Although the weight loss increased in parallel with the increasing duration of PRESI, the difference was not significant. These results were expected because an exposure to a heat-treatment and 10 days storage could speed up the water evaporation from the egg. The same results were reported by *Petek and Dikmen (2006)* and *Jotanović et al. (2010).*

PRESI treatments significantly affected the hatchability of total and fertile eggs. Hatchability of total eggs was significantly lower in 9h PRESI group indicating that prestorage incubation longer than 6 h had a depressive effect on the vitality of embryos. This result is in accordance to previous reports form *Silva et al. (2008)* who reported that PRESI for 6 h improves hatchability and *Jotanović et al. (2010)* who found a significant reduction of hatchability in eggs exposed to PRESI for 12h. *Fasenko et al. (2001a)* reported that the benefits of PRESI appear to be storage duration dependent and suggested that a 6-h PRESI is optimum.

Table 2. The effect of prestorage incubation on embryonic mortality, %

Incubation period	Duration of prestorage incubation			
	0	3h	6h	9h
1-7 days	5.17^{ab}	2.31^{b}	4.62^{ab}	8.85^{a}
8-14 days	1.10	1.53	0.77	1.15
15-21days	2.21^{b}	4.23^{a}	4.23^{a}	3.85^{ab}
Total	8.49^{ab}	8.08^{b}	9.62^{ab}	13.84^{a}

a-b Values within the rows with no common superscript are significantly different (P<0.05)

There were significant differences between treatments regarding to embryonic mortality. Eggs in groups exposed to 9h of PRESI had the highest embryonic mortality in the first week of incubation. That was the main cause of increased total embryonic mortality in this group of eggs. *Jotanović et al. (2010)* reported that early embryonic mortality was very high (18.84%) in 9 h PRESI groups and even 30.19 % in 12 h PRESI groups. *Fasenko et al. (2010 a)* found that early embryonic mortality in eggs exposed to 18 h PRESI and stored for 14 days was significantly increased compared to the other groups with shorter PRESI duration. This was also highly dependent on storage duration.

Conclusion

In conclusion, PRESI of commercial broiler breeder eggs has a potential to be used by the producers as a method to improve hatchability of eggs stored for longer than 7 days. Further research is needed to more precisely determine the number of hours of PRESI required to obtain maximum hatchability. Based on the results of this trial PRESI treatment shouldn't be longer than 6 hours. However, it should be emphasized that the benefits of PRESI appear to be storage duration dependent and different egg storage durations could alter the effectiveness of the PRESI treatments.

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Efekat kratkotrajne inkubacije nasadnih jaja pre dužeg skladištenja na rezultate izvođenja

L. Perić, D. Žikić, M. Đukić Stojčić

Rezime

Ovaj rad je baziran na konceptu da će kratkotrajna inkubacija nasadnih jaja pre skladištenja (PRESI) imati pozitivan uticaj na preživljavanje embriona tokom dugotrajnog skladištenja. Ukupno 1120 nasadnih jaja brojlerskih roditelja sakupljeno je sa komercijalne farme i podeljeno je u 4 grupe koje predstavljaju 4 perioda zagrevanja: 0 h – kontrolna grupa, 3 h PRESI, 6 h PRESI i 9 h PRESI.

Posle inkubacije u trajanju od 3, 6 ili 9 sati, jaja su izvađena iz inkubatora i ostavljena u prostoriju za skladištenje na temperaturi od 16 °C i relativnoj vlažnosti vazduha od 65-75%. Skladištenje je trajalo 10 dana. Nakon toga, sva jaja su uložena u inkubator i dobijeni su sledeći rezultati: prosečan procenat leženja kod kontrolne grupe iznosio je 85.21%, kod 3 h PRESI 87.69 %, kod 6 h PRESI 83.46% i kod 9 h PRESI 79.61%. Iz dobijenih rezultata može se zaključiti da PRESI tretman u trajanju od tri sata ima pozitivan uticaj na izvođenje pilića iz jaja koja su skladištena 10 dana, dok PRESI tretman u trajanju od 9 sati značajno smanjuje procenat izvođenja.

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THE INFLUENCE OF THE LOHMANN BROWN LAYING HENS' AGE ON EGG WEIGHT AND STRUCTURE

T. Pandurevic[1], S. Mitrovic[2], V. Djermanovic[2], V. Djekic[3], M. Lalovic[1]

[1]Faculty of Agriculture, University of East Sarajevo, Vuka Karadzica 30, 71123 East Sarajevo, Republic of Srpska, Bosnia and Herzegovina.
[2]University of Belgrade, Faculty of Agriculture, Institute of Zootechnic, Nemanjina 6, 11080 Zemun, Republic of Serbia.
[3]Center for Small Grains, Save Kovacevic 31, 34000 Kragujevac, Republic of Serbia.
Corresponding author: djermanovic@agrif.bg.ac.rs
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Abstract: In order to determine the influence of the Lohmann Brown laying hens' age on egg weight and structure (the shares of eggshell, albumen and yolk) and their phenotypic correlation relationship, an experiment has been carried out on four egg samples, originating from the laying hens of different age: (AH_{20}), 28 (AH_{28}), 49 (AH_{49}) and 72 weeks (AH_{72}). The average weight of eggs was increasing statistically significantly ($P<0.001$) with the ageing of the laying hens, so that at the beginning of the production cycle it was 46.15 g (AH_{20}), and in the end it was AH_{72} 69.11 g. Also, the absolute share of eggshell, albumen and egg yolk in the egg weight was statistically significantly higher ($P<0.001$) with elderly laying hens than with the younger ones. The percentage share of the eggshell in the egg weight was gradually decreasing with the ageing of the laying hens from 13.41% (AH_{28}) to 11.78% (AH_{72}). All of the differences were statistically significant ($P<0.001$) except the difference (0.07%) between AH_{49} and AH_{72} which was not statistically significant ($P>0.05$). The share of yolk in the egg weight indicated a similar decreasing trend, so that in the first half of the production cycle it ranged between 23.60% (AH_{20}) and 25.90% (AH_{28}), and in the second half of the egg production between 24.49% (AH_{49}) and 24.25% (AH_{72}). Unlike the share of yolk, the share of albumen in the egg weight was similar, but positive, and it ranged between 60.69% (AH_{28}) and 63.97% (AH_{72}). The ageing of the laying hens statistically significantly ($P<0.001$) influenced on the decrease (the yolk), i.e. increase (egg white) of the shares of the yolk and albumen in the egg weight, except between AH_{49} and AH_{72} when the differences determined have not been statistically confirmed ($P>0.05$).

Key words: Lohmann Brown, laying hens, age, egg wight, eggshell, yolk, albumen, correlation.

Introduction

The quality of table eggs is influenced by the different factors among which genotype, the age of the laying hens and oviposition take significant place. Some authors, such as *Ledvinka et al. (2000)*, *Leyendecker et al. (2001a)* and *Vits et al. (2005)*, state that laying hens, which lay eggs of brown color of the eggshell, carry, as a rule, the larger eggs, while *Leyendecker et al. (2001b)* and *Zhang et al. (2005)* found a bigger share of yolk in the egg of white color eggshell, and *Harmann et al. (2000)* in the eggs of the brown color eggshells. *Peebles et al. (2000)*, *Silversides and Scott (2001)*, *Suk and Park (2001), Van de Brand et al. (2004)*, *Rizzi and Chiericato (2005)*, *Campo et al. (2007), Johnston and Gous (2007)*, *Rajičić et al. (2007), Zemkova et al. (2007), Lukaš et al. (2009)*, *Mitrovic et al. (2010)* have established, in their researches, that age of laying hens influences on the quality egg. Researches of the some authors verify that the egg weight, beside the age of laying hens, also influences on the absolute and percentage shares of the eggshell, egg white, yolk and other characteristics of the quality of an egg *(Zhang et al., 2005; Djekić et al., 2008; Shi et al., 2009; Lukaš et al., 2009; Mitrovic et al., 2010)*. Then, in similar research conducted some interesting studies *(Tumova and Ebeid, 2005; Tumova and Ledvinka, 2009; Tumova et al., 2009)*. They have examined the influence of the system of housing (in cage and on floor) of the laying hens and oviposition on the quality of eggs in the cases of different genotypes of hens and they stated that the housing system has less impact on the weight and the quality of eggs, and that the time of the collection of eggs had more significant impact, particularly in the cage system of housing laying hens.

The aim of this study was somewhat similar to the previous researches, including that some properties of the quality of eggs have been examined in the cases of laying hens of Lohmann Brown hybrids at different ages, as follows: 20 weeks old laying hens (the initial stage of egg production), then in 28 weeks old laying hens (the maximum intensity of load), in 48 weeks old laying hens (the middle of the egg production) and at the end of the production cycle when the laying hens were 72 weeks old. Besides that, by calculating the coefficient of the phenotypic correlation between the observed parameters in a certain age of laying hens, and for the whole production cycle, the intensity of the correlation connection between the age of the laying hens, i.e. egg weight and a relative share of raw eggshell, egg white and yolk, has been determined.

Material and Methods

In order to establish the influence of the age of Lohmann Brown laying hens on egg weight and structure, some researches have been conducted on altogether

640 eggs. During the period of egg production from 20th to 72nd week of laying hens' age, by 160 eggs were taken for analysis by the method of random sample at different age of laying hens. The sampling of eggs was performed at the beginning of the production cycle, when the laying hens were 20 weeks old (AH_{20}), then at the maximal production of eggs when the laying hens were 28 weeks old (AH_{28}), then when the laying hens were 49 weeks old (AH_{49}), and in the end of the production cycle (AH_{72}). Lohmann Brown laying hens were grown in cages on farm LTD Rakic Commerce, located in Republic Srpska, Bosnia and Herzegovina. At different age of the laying hens, altogether 640 eggs were taken by the method of random sample for four times during the production cycle. These 640 eggs were packed and transported to experimental laboratory of Faculty of Agriculture in Zemun. The eggs were individually measured in the laboratory. In order to establish the structure, each egg was broken after measurement, and then, raw eggshell, yolk and egg white were measured. The following parameters were established at all of the four groups of eggs, egg weight (g), the share of the raw eggshell (g, %), egg white (g, %) and yolk (g, %).

The common variation statistical indicators are calculated for all indicators: average values, the error of the arithmetical mean, standard deviation and coefficient of the variation. The test of the significance of the differences between the analyzed characteristics was carried out by the applying an appropriate model of variance analysis with an equal number of repetition per treatments. Mathematical variance analysis model was as follows:

$$Y_{ij} = \mu + AH_i + e_{ij}$$

Y_{ij} – individual of i – e age i = 1,2,...; μ - the general population everage; AH_i – impact of the i-th age period, i = 1 (AH_{20}), 2 (AH_{28}), 3 (AH_{49}), 4 (AH_{72}); e_{ij} – random error.

All significant differences established on the basis of applied analyses of the variance and the results of the F-exp value have been marked by the Tukey test. The coefficients of the phenotypic correlation relationship between the egg weight and the observed characteristics of the egg structure have been calculated for the above mentioned phases and the whole production cycle. Statistical data processing was done using SAS/STAT *(SAS Institute, 2000)*.

Results and Discussion

The data presented in the Tables 1 and 2 verify that the age of laying hens during the production cycle affect the egg weight, absolute and relative share of eggshell, albumen and yolk in the egg weight.

Table 1. Average values and variability of egg weight and egg structure

Traits	Age of hens (weeks)	n	$\bar{x}$	$S_{\bar{x}}$	S	C.V.
Eggs weight, g	AH_{20}	160	46.15	0.34	4.27	9.25
	AH_{28}	160	59.89	0.33	4.12	6.88
	AH_{49}	160	67.12	0.36	4.62	6.88
	AH_{72}	160	69.11	0.42	5.27	7.63
	Total	640	60.57	0.40	10.10	16.67
Eggshell, g	AH_{20}	160	6.12	0.06	0.79	12.95
	AH_{28}	160	8.03	0.06	0.72	8.97
	AH_{49}	160	7.93	0.03	0.42	5.30
	AH_{72}	160	8.11	0.04	0.49	6.04
	Total	640	7.55	0.04	1.07	14.17
Albumen, g	AH_{20}	160	29.16	0.24	3.08	10.56
	AH_{28}	160	36.40	0.25	3.17	8.71
	AH_{49}	160	42.79	0.32	4.03	9.42
	AH_{72}	160	44.28	0.33	4.18	9.44
	Total	640	38.16	0.26	6.63	17.37
Yolk, g	AH_{20}	160	10.88	0.11	1.45	13.33
	AH_{28}	160	15.47	0.05	0.67	4.33
	AH_{49}	160	16.40	0.06	0.75	4.57
	AH_{72}	160	16.72	0.07	0.85	5.08
	Total	640	14.87	0.10	2.57	17.28
Eggshell, %	AH_{20}	160	13.27	0.11	1.36	10.25
	AH_{28}	160	13.41	0.07	0.86	6.41
	AH_{49}	160	11.85	0.06	0.76	6.41
	AH_{72}	160	11.78	0.05	0.66	5.60
	Total	640	12.58	0.05	1.22	9.70
Albumen, %	AH_{20}	160	63.13	0.17	2.16	3.42
	AH_{28}	160	60.69	0.17	2.12	3.49
	AH_{49}	160	63.66	0.15	1.87	2.94
	AH_{72}	160	63.97	0.12	1.57	2.45
	Total	640	62.86	0.09	2.33	3.71
Yolk, %	AH_{20}	160	23.60	0.13	1.67	7.08
	AH_{28}	160	25.90	0.11	1.36	5.25
	AH_{49}	160	24.49	0.09	1.12	4.57
	AH_{72}	160	24.25	0.09	1.10	4.54
	Total	640	24.56	0.06	1.57	6.39

Table 2. The significance of the differences in egg structures in different stages of the production cycle

Traits	Age of hens (weeks)	$\bar{x}$	d	Significance
Eggs weight, g	$AH_{20} – AH_{28}$	46.15 – 59.89	-13.74	***
	$AH_{20} – AH_{49}$	46.15 – 67.12	-20.97	***
	$AH_{20} – AH_{72}$	46.15 – 69.11	-22.96	***
	$AH_{28} – AH_{49}$	59.89 – 67.12	-7.23	***
	$AH_{28} – AH_{72}$	59.89 – 69.11	-9.22	***
	$AH_{49} – AH_{72}$	67.12 – 69.11	-1.99	***
Eggshell, %	$AH_{20} – AH_{28}$	13.27 – 13.41	-0.14	ns
	$AH_{20} – AH_{49}$	13.27 – 11.85	1.42	***
	$AH_{20} – AH_{72}$	13.27 – 11.78	1.49	***
	$AH_{28} – AH_{49}$	13.41 – 11.85	1.56	***
	$AH_{28} – AH_{72}$	13.41 – 11.78	1.63	***
	$AH_{49} – AH_{72}$	11.85 – 11.78	0.07	ns
Albumen, %	$AH_{20} – AH_{28}$	63.13 – 60.69	2.44	***
	$AH_{20} – AH_{49}$	63.13 – 63.66	-0.53	*
	$AH_{20} – AH_{72}$	63.13 – 63.97	-0.84	***
	$AH_{28} – AH_{49}$	60.69 - 63.66	-2.97	***
	$AH_{28} – AH_{72}$	60.69 – 63.97	-3.28	***
	$AH_{49} – AH_{72}$	63.66 – 63.97	-0.31	ns
Yolk, %	$AH_{20} – AH_{28}$	23.60 – 25.90	-2.30	***
	$AH_{20} – AH_{49}$	23.60 – 24.49	-0.89	***
	$AH_{20} – AH_{72}$	23.60 – 24.25	-0.65	***
	$AH_{28} – AH_{49}$	25.90 – 24.49	1.41	***
	$AH_{28} – AH_{72}$	25.90 – 24.25	1.65	***
	$AH_{49} – AH_{72}$	24.49 – 24.25	0.24	ns

* $P<0.05$, *** $P<0.001$, ^{ns} $P>0.05$.

The data in the Table 1 show that the egg weight was increasing together with the age of the laying hens, so that, at the beginning of the production cycle, (AH_{20}) amounted to 46.15 g, and at the end, (AH_{72}) the egg weight amounted to 69.11 g, while the average egg weight for the whole production cycle was 60.57 g. The established differences in egg weight were statistically significant ($P<0.001$) among all of the phases of the production cycle, i.e. among different age of laying hens (AH_{20}, AH_{28}, AH_{49}, AH_{72}) Table 2.

The data from the Table 1 and Table 2 reveal further that the average values of the relative share of eggshell were decreasing with the increase of the laying hens' age, except at the maximum intensity of egg load when the share of the eggshell in the egg weight was highest, amounting to 13.41%. From the abovesaid, it arises that the share of the eggshell of the eggs originating from the older laying hens was statistically significantly ($P<0.001$) lower except in the first eight weeks of the production, as well as between AH_{49} and AH_{72}, where the difference was not statistically confirmed ($P>0.05$). To the contrary of the eggshell, the share of the

albumen in the egg weight revealed the tendency of increase with the increasing of the laying hens' age. However, it should be emphasized that the share of the albumen at the beginning of the production cycle (AH_{20} - 63.13%) was statistically significantly higher ($P<0.001$) than at the maximal egg production (AH_{28} – 60.69%). In the second phase of the production cycle (AH_{49} and AH_{72}) the albumen share was approximately equal, and the difference established was not statistically significant ($P>0.05$). At the maximal intensity of egg load (AH_{28}) the share of the yolk in the egg weight was highest, and the lowest at the beginning of the egg production period (AH_{20}). The differences discovered were statistically significant ($P<0.001$), except the difference in the second phase of the production cycle, which was not statistically confirmed ($P>0.05$).

Most of the authors *(Peebles et al., 2000; Silversides and Scott, 2001; Van de Brand et al., 2004; Rizzi and Chiericato, 2005; Johnston and Gous, 2007; Lukaš et al., 2009; Mitrovic et al., 2010)*, who were testing the effect of the laying hens' age on the egg weight and structure at different breed and hybrid of the hens, have concluded that the egg weight statistically significantly ($P<0.01$) increases with the increase of the laying hens' age, except in the paper where is conclusion that the age of laying hens does not affect statistically significantly ($P>0.05$) the egg weight *(Zemkova et al., 2007)*. The authors state that the age of the laying hens too, besides the genotype, significantly affects the quality of eggs. Regardless of the genotype, the egg weight and the percentage of yolk in egg weight were increasing together with the increase of laying hens' age in case of all hens while the percentage of the eggshell and the albumen in egg weight were decreasing. Unlike these researches, in our study, the percentage share of yolk in the egg weight was insignificantly lower in the case of older laying hens (AH_{49} and AH_{72}), but the difference was not statistically significant ($P>0.05$).

All the above presented indicates to the fact that there is a certain connection between the age of the laying hens and the egg weight, as well as between some quality characteristics, i.e. egg structures. In addition to this, the coefficients of phenotypic correlation between the weight of eggs originating from four groups of laying hens (AH_{20}, AH_{28}, AH_{49} i AH_{72}) and the relative shares of the eggshell, albumen and yolk (Table 3) have been calculated.

Table 3. The coefficients of phenotypic correlation between the egg weight (g) and the shares of eggshell, albumen and yolk (%)

Traits	r_{20}	r_{28}	r_{49}	r_{72}	r_{total}
Eggshell	-0.425^{***}	-0.142^{ns}	-0.786^{***}	-0.665^{***}	-0.614^{***}
Albumen	0.086^{ns}	0.534^{***}	0.754^{***}	0.688^{***}	0.318^{***}
Yolk	-0.093^{ns}	-0.776^{***}	-0.701^{***}	-0.639^{***}	-0.039^{ns}

*** $P<0.001$, ^{ns} $P>0.05$.

In the case of three groups (AH_{20}, AH_{49}, AH_{72}) of laying hens of different age, the negative statistically significant ($P<0.001$) coefficient of phenotypic correlation have been established between the egg weight and percentage share of eggshell, except the coefficient AH_{28}. Similarly, the statistically significant ($P<0.001$) negative coefficients of phenotypic correlation (AH_{28}, AH_{49}, AH_{72}) between the egg weight and the share of the yolk have been established, except in the case of eggs originating from the laying hens from the beginning of the egg production (AH_{20}), where the coefficient established was not significant ($P>0.05$). The coefficients of the correlation between the albumen and the egg weight, unlike the yolk, were statistically significantly ($P<0.001$) positive (AH_{28}, AH_{49}, AH_{72}), i.e. positive (AH_{20}), but not statistically significant ($P>0.05$).

A similar phenotypic correlation relationship between the egg weight and the shares of the eggshell, albumen and yolk was established *(Zhang et al., 2005; Rajičić et al., 2007; Djekić et al., 2008; Mitrovic et al., 2010; Pandurevic, 2011)*. Similar researches have conducted in the case of eggs of the average weight 54.376 g divided in the three weight groups *(Shi et al., 2009)*. The established coefficients of correlation and their significance we came to in our researches, too, were approximately similar, especially in the case of eggs originating from the 28-weeks-old hens. Somewhat different correlation relationship between the egg weight and share of the eggshell, albumen and yolk was established by a negative correlation relationship between the egg weight and the percentage share of eggshell and albumen, and a positive one between the egg weight and the percentage share of yolk by some authors *(Lukaš et al., 2009)*.

Conclusion

It has been established by the researches that, together with the age of Lohmann Brown laying hens, the egg weight and the absolute share of raw eggshell, albumen and yolk in the egg weight, statistically significantly ($P<0.001$) increase. Besides that, the relative shares of raw eggshell, and yolk have shown the tendency of decrease with the increase of laying hens' age, while the share of the albumen have shown the tendency of increase with the increase of laying hens' age. This statement is verified by the calculated coefficients of phenotypic correlation between the laying hens age i.e. the egg weight and the shares of the structural parts of an egg.

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Uticaj starosti kokoši nosilja Lohmann Brown na težinu i strukturu jaja

T. Pandurevic, S. Mitrovic, V. Djermanovic, V. Djekic, M. Lalovic

Rezime

U cilju utvrđivanja uticaja starosti kokoši nosilja Lohmann Brown na težinu i strukturu jaja (udeo ljuske, belanceta i žumanceta) i njihove fenotipske korelacione povezanosti sproveden je eksperiment na četiri uzorka jaja poreklom od kokoši nosilja različite starosti: 20 nedelja (AH_{20}), 28 (AH_{28}), 49 (AH_{49}) i 72 nedelje (AH_{72}). Sa starošću nosilja povećavala se statistički značajno ($P<0.001$) prosečna težina jaja tako da je na početku proizvodnog ciklusa iznosila 46.15 g (AH_{20}), a na kraju (AH_{72}) 69.11 g. Isto tako, apsolutni udeo ljuske, belanceta i žumanceta u težini jajeta je bio statistički značajno veći ($P<0.001$) kod starijih, nego kod mlađih kokoši nosilja. Procentualni udeo ljuske u težini jajeta se sa starošću nosilja postepeno smanjivao sa 13.41% (AH_{28}) na 11.78% (AH_{72}). Sve razlike su bile statistički signifikantne ($P<0.001$), osim razlike (0.07%) između AH_{49} i AH_{72} koja nije bila statistički značajna ($P>0.05$). Sličan trend smanjenja, pokazao je i udeo žumanceta u težini jajeta, tako da se u prvoj polovini proizvodnog ciklusa kretao između 23.60% (AH_{20}) i 25.90% (AH_{28}), a u drugoj polovini produkcije jaja između 24.49% (AH_{49}) i 24.25% (AH_{72}). Za razliku od udela žumanceta, udeo belanceta u težini jajeta bio je sličan, ali pozitivan, i kretao se između 60.69% (AH_{28}) i 63,97% (AH_{72}). Starost nosilja je statistički signifikantno ($P<0.001$) uticala na smanjenje (žumance), odnosno povećanje (belance) udela žumanceta i belanceta u težini jajeta, osim između AH_{49} i AH_{72} kada utvrđene razlike nisu statistički potvrđene ($P>0.05$).

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MANURE TREATMENT WITH RED EARTHWORMS *(Lumbricus rubellus)* AND POSSIBILITY OF USE OF BIOMASS FOR POULTRY FEED

Lj. Janković[1], B. Radenković-Damnjanović[1], M. Vučinić[1], Š. Pintarič[2]

[1]Faculty of Veterinary Medicine, Bulevar oslobođenja 18, Belgrade, Republic of Serbia
[2]Faculty of Veterinary Medicine, Gerbičeva 60, Ljubljana, Slovenia
Corresponding author: lili@vet.bg.ac.rs
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Abstract: The aim of this study was to evaluate the nutritional value of biomass of red earthworms (*Lumbricus rubellus*) and powder made of these, as well as the impact of nutrition mixtures in which the fish meal was replaced by worm meal and fresh earthworms on the health and performance of broilers. The results of chemical analyzes showed that red worm meal contained 41.42% protein (DM) and satisfactory amounts of amino acids. The experiment was performed on 100 Hybro broiler chickens divided into four groups and lasted 42 days. Broilers of the control group were given feed made of standard components and chemical composition, whilst 50 or 100% fish meal was replaced with worm powder in the meal of the first and second experimental group, respectively. The third experimental group was fed without fish meal, but was allowed to consume fresh earthworms ad libitum. At the end of the first phase of fattening the broilers in the third experimental group reached significantly lower body weight ($p<0.05$) compared to the control. At the end of the second and third phase, as well as regarding the whole experiment, the difference in body weight of broilers consuming fish meal and those consuming worm powder was insignificant ($p>0.05$). In addition, the differences in daily weight gain between the groups were not significant ($p>0.05$).

Key words: manure, red earthworm (*Lumbricus rubellus*) powder, body weight, broiler chickens

Introduction

Apart from being producers of food for human consumption, livestock farms are sources of huge amounts of waste, primarily manure. The use of manure to maintain soil fertility carries a number of environmental risks *(Vuković et al., 2000; Janković et al., 2000; 2003; 2004; 2007; Đorđević et al., 2006)*. In recent years

manure is being processed by red earthworms. During this procedure, in addition to high-quality organic fertilizer - humus, large amounts of lumbriculture biomass are produced. Humus is more or less homogeneous with desirable aesthetic properties, reduced content of pollutants, high nutritional content which does not change for a long period and has no negative impact on the environment *(Ndegwa et al., 2000; Janković et al., 2002)*.

Biomass, whether fresh or dry, can serve as an alternative source of proteins in the feed for fish and non-ruminants *(Cilevski, 1994; Zhen-Jun, 1995; Đorđević et al., 2008; Janković, 2006; Nguyen and Ulfert, 2009)*.

Given the progressive increase in livestock, it is estimated that the protein deficiency in animal feed will reach approximately 2.000,000 tons and that only in EU about 2.000,000 tons of soybean meal will be necessary *(Pearse, 2001)*. Using the existing methods for extraction and preparation of powder, about 25-50% of the protein could be provided from earthworm meal.

For manure processing two worm species of the family Lumbricidae are suitable: *Lumbricus rubellus* and *Eisenia foetida*. Under optimal conditions (temperature of 18-22^{0}C, manure humidity 80%, pH 7 and food) worms reproduce quickly. The primary food of the worm is fermented bovine, equine, swine and sheep manure. Manure which is given to worms to feed must undergo fermentation as some Salmonella species (*S. enteritidis, S. gallinari, S. typhimurium, S. anatum, S. cairo*) can survive up to 345 days in solid manure, and it may contain various parasites transmitted by worms, including developmental stages of protozoa, cestodes and nematodes *(Elvira et al., 1996)*.

Worms are hermaphrodites. They breed cocoons from which young worms emerge after 2-3 weeks and sexually mature after about 90 days (*Lumbricus rubellus*). Worms reach full length at the age of 10 months. They breed quickly, their number increasing by geometric progression, so 2 beds after 3 months result in 4 litters with about 100,000 individuals. One adult sexually mature individual consumes about 1g of food per day. About 60% of processed food is converted into humus *(Edwards et al., 1995)*. The transformation of manure by earthworms is relatively uniform: from 100 kg of cattle manure between 0.9 and 2.6 kg of earthworms and 49-57 kg humus can be obtained. One litter (population of about 100,000 worms) during the annual cycle of production (9-10 months) outdoors processes 1 ton of manure *(Ndgewa et al., 2000)*.

Earthworm biomass occurred during manure processing contains a complex of biologically active substances and can be used as feed for fish and non-ruminant species, either fresh or processed. Powder obtained from dried worms is characterized by high protein content (55.97%-63.4%) and a favorable amino acid composition *(Nadaždin et al., 1988; Cilevski, 1994; Paoletti et al., 2003; Nguyen et al., 2009)*. Due to a relatively high percentage of fat in comparison to other feed,

Californian worms are a rich source of energy *(Zhen-Jun, 1995)*. *Dynes (2003)* found that the lipid content in earthworms varies between 1% and 20% of dry matter and that its composition is similar to that of fish oil.

It was experimentally proved that worm powder is a good source of proteins for broilers and that besides fresh worms it is a very tasty feed *(Cilevski, 1994; Zhen-Jun, 1995)*. *Ulep (1982)*states that at any stage of broiler chicken production worm meal may replace fish, meat and soybean meal, resulting in slightly lower broiler body weight. *Das and Dash (1990)* and *Nguyen et al. (2009)*report that the weight gain of broiler chickens fed with fresh earthworms was significantly higher compared with the control, whilst *Cilevski (1994)* and *Zhen Jun (1995)* reported data on higher daily weight gain in the first phase, and a slightly lower in the second phase of fattening, with relatively uniform feed intake and conversion between the groups.

In recent years, poultry production has been expanding due to the fact that poultry meat production is comparatively faster and less expensive. It is estimated that the production and consumption of poultry meat will continue to increase because in addition to good feed conversion and attractive sensory quality traits, it is characterized by low fat content and high levels of protein, so it is favorable from nutritional and medical viewpoints. One of the prerequisites for the production of sufficient quantities of manufactured poultry feed is the provision of sufficient quantities of protein sources of animal origin. These nutrients are provided mainly by import because domestic production is both insufficient and expensive. For this reason, the possibilities of their replacement and the use of nutrients of domestic origin in solving the problem of insufficient high-value protein for animal feed are constantly being explored.

The aim of our study was to examine the quality of the earthworm *Lumbricus rubellus* biomass, as well as the possibility of its use as an alternative protein source in fattening broilers, which was monitored by the production performance and health.

Material and Methods

The research was performed on 100 one-day-old Hybro chickens divided into four equal groups. The experiment lasted 42 days and passed in three phases: the first phase lasted three, the second two and third one only one week.

Body mass and food consumption were measured at the end of each phase of the experiment and at the same time feed samples were taken for chemical analysis. At the end of the experiment, on day 42, the broilers were measured and slaughtered. Meat was sampled for analysis after primary processing and cooling.

A control group of broilers were fed with a complete feed mixture for fattening chickens, containing standard components and of standard chemical composition (FSH production "Inshra"). For groups I and II 50% and 100% fish meal was substituted with worm powder, respectively; the III experimental group received feed with no meal, but was given fresh, chopped earthworms ad libitum from day one to the end of the experiment (Table 1). Earthworm powder was made from the earthworm species *Lumbricus rubellus* purchased from the producer "Lumbri bio agrar", and then dried in the oven 5 hours at 55^0C. Upon completion of drying, the worms were milled into powder. The resulting meal was light brown in color and had pleasant aroma.

Broiler feed components were mixed in the technology laboratory at the Faculty of Technology in Novi Sad in a mixer with a capacity of 50 kg. The mixing lasted 25 minutes and resulted in a homogeneous mass. Broilers were fed complete mixtures for initial fattening from day 1 to 21, grower from day 22 to 35 and finisher from day 36 to the end of the experiment (Table 1).

Table 1. Composition of diets in each experimental phase (%)

Ingredients [%]	Exsperimental phase											
	Days 01-21				Days 22-35				Days 36-42			
	C	E-I	E-II	E-III	C	E-I	E-II	E-III	C	E-I	E-II	E-III
Maize	55.7	53.8	51.9	60.7	62.4	61.2	60.0	65.4	66.0	66.0	66.0	66.0
Soybeen meal	26.0	26.0	26.0	26.0	21.0	21.0	21.0	21.0	21.0	21.0	21.0	21.0
Sunflower pellets	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0	5.0
Yeast	3.0	3.0	3.0	3.0	3.0	3.0	3.0	3.0	2.0	2.0	2.0	2.0
Fish meal	5.0	2.5	-	-	3.0	1.5	-	-	-	-	-	-
Earthworm meal	-	4.0	8.0	-	-	2.5	5.0	-	-	-	-	-
Vegetable oil	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Dicalcium phosphate	1.0	1.2	1.6	1.6	1.0	1.1	1.2	1.2	1.1	1.1	1.1	1.1
[1]Chalk	0.6	0.8	0.9	0.6	0.8	0.8	0.8	0.8	1.1	1.1	1.1	1.1
Salt	0.2	0.2	0.2	0.2	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
[2]VMP	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Total	100	100	100	100	100	100	100	100	100	100	100	100

Sampling of fresh worms, worm meal and dehydrated complete broiler feed was performed with standard procedures and methods *(Pravilnik, 4/2010)*, and the analysis with contemporary methods.

Basic nutrients were determined following the modified procedure described by Weende *(quoted by Sinovec and Ševković, 1995)*, and the determination of calcium and phosphorus using the colorimetric method on the appliances produced by Tecator System. The calculation of metabolic energy was based on the results of the nutrient content, using the appropriate formula *(Grbeša, 2004)*. The content of each amino acid in proteins was determined with the chromatographic method on an automatic amino-analyzer.

The extraction of lipids from the worm meal was carried out by a modified Folch-Lees's method *(Folch et al., 1957)* and followed by their hydrolysis into fatty acids and the esterification of these into methyl esters. Fatty-acid composition analysis was performed by the gas-chromatographic method using standard fatty acid methyl esters ("Sigma", USA) on the gas chromatograph Hewlett Packard HP6890.

The heavy metal (Pb, Cd, Hg, As) content in the worm meal was determined by flame atomic absorption spectro-photometry on the device "Varian Spectra 220" as recommended by the *Analytical Chemistry Laboratory Guidebook (1991)*. Pb and Cd were determined by the flame technique, and Hg and As on the device "VGA 77" using the cold vapor technique and hydride technique, respectively. The usability of the meal was assessed according to objective criteria *(Pravilnik o maksimalnim količinama štetnih materija i sastojaka u stočnoj hrani, Sl. list SFRJ br. 2/90 i 27/90)*.

The samples of fresh worms and worm meal were taken according to standard procedures *(Pravilnik, 4/2010)* and analyzed with standard microbiological techniques of isolation and identification of microorganisms and determination of the total number of bacteria and fungi *(Manual for Laboratory Diagnosis - standardization of diagnostic methods for bacterial, viral and parasitic diseases whose prevention is required by law, 1984)*. The assessment of the usability was based on objective criteria *(Pravilnik o maksimalnim količinama štetnih materija i sastojaka u stočnoj hrani, Sl. list SFRJ br. 2/90 i 27/90)*.

In order to monitor the production performance, the body mass of broilers was measured individually at the beginning of the experiment and at the end of its each phase on an electronic scale with an accuracy of 10^{-2}g. The average body weight of broilers was calculated at the end of each phase, as well as for the entire essay. In addition, total and daily weight gains were calculated.

Throughout the entire experiment the amount of complete diets consumed by each experimental group, and the amount of fresh worms consumed by group III

were measured. Total feed intake was measured at the end of the experiment and its each stage.

The data obtained were processed with statistical methods. Measures of variation and correlation coefficients were calculated, ANOVA performed and followed by the F test and Tuckey test, and the regression equation derived.
All tests were used at the risk of 5% and 1%, and therefore the conclusions are given with the corresponding probability (95 and 99%).

Results and Discussion

The results of the analysis of the chemical composition of fresh earthworms (*Lumbricus rubellus*) and dehydrated earthworm meal are shown in Table 2. There are the average values obtained from individual earthworm samples analyzed at intervals given in Material and methods.

Table 2. Chemical composition of fresh worms, earthworm meal and fish meal (% DM)

Chemical composition	Fresh worms	Earthworm meal	Fish meal
Moisture	84.76	11.44	10.00
Ash	1.32	9.20	19.57
Protein	6.89	41.42	67.39
Fat	2.25	9.20	7.17
Fibre	0.55	1.77	0.65
[1]FNE	4.14	25.00	5.22
[2]ME, MJ/kg		10.64	14.18
Ca	0.20	1.46	7.28
P	0.14	0.80	3.48
Lysine	0.51	3.33	5.11
Methionine	0.14	0.96	1.96

[1] FNE- free nitrogen extract;
[2] ME - metabolic energy

It is noticeable that fresh red earthworms on extracting from the litter contain 6.89% protein, whilst worm meal obtained by drying contains 41.42% protein. In the process of obtaining worm meal there is an increase in ash, calcium, phosphorus and fat contents. There is also a difference in the chemical composition of fresh earthworms on sampling and after storage in water for 24 hours.

The results of fatty acid contents in dehydrated worm powder are given in Table 3. The presence of the following fatty acids was detected: palmitic (C16:0), palmitoleic (C16:1), stearic (C18:0), oleic (C18:1), linoleic (C18:2), linolenic (C18:3) and arachidic acid (C20:0).

Table 3. Fatty acid contents in redworm powder (%)

Fatty acids				Measures of variation		
	$\bar{x}$	±	Sd	Iv	Se	Cv
C16:0	11.52		0.09	11.39-11.65	0.04	0.82
C16:1	6.24		0.04	6.18-6.29	0.02	0.67
C18:0	6.26		0.04	6.20-6.31	0.02	0.60
C18:1	12.87		0.37	12.14-13.14	0.15	2.85
C18:2	4.83		0.06	4.77-4.91	0.02	1.15
C18:3	0.55		0.02	0.52-0.58	0.01	3.64
C20:0	0.15		0.01	0.14-0.17	0.00	7.71

The content of heavy metals is shown in Table 4. It can be noticed that lead, cadmium and mercury were not detected, and the amounts of arsenic (0.59 mg/kg) were within the limits prescribed by the Regulations on maximum levels of harmful substances and ingredients in animal feed *(Sl. list SFRJ 2/90 i 27/90)*.

Table 4. Heavy metal content in earthworm meal (mg/kg)

Heavy metal	Content (mg/kg)	Maximum allowed content (MAC)
Pb	0.00	10.00
Cd	0.00	0.50
Hg	0.00	0.20
As	0.59	2.00

The results of the microbiological analysis of fresh worms and dehydrated worm meal are shown in Table 5. It can be seen that both worm and meal samples meet the required standards of hygiene *(Pravilnik o maksimalnim količinama štetnih materija i sastojaka u stočnoj hrani, Sl. list SFRJ br. 2/90 i 27/90)*.

Table 5. Microbiological analysis of samples of fresh earthworms and earthworm meal

Microorganism	Samples of fresh earthworms	Samples of earthworms meal
Bacillus spp.	+	+
Stapylococcus spp.	+	+
Pseudomonas spp.	+	+
Coliform bacteria	+	+
Total no. of bacteria	9.0×10^6/g	1.8×10^6
Mucor spp.	+	+
Aspergillus spp.	+	+
Penicillium spp.	+	+
Total no. of fungi	36×10^3	1×10^3

The chemical composition of complete feed mixtures for broilers is shown in Table 6. It can be seen that it fully satisfied the needs of broilers in each stage of fattening and matched the requirements of the experiment.

Table 6. Chemical composition of feed mixture for broilers in each experimental phases (%)

Chemical composition	Feed mixture I (01-21 days)				Feed mixture II (22-35 days)				Feed mixture III (36-42 days)			
	C	E-I	E-II	E-III	C	E-I	E-II	E-III	C	E-I	E-II	E-III
Moisture	11.11	11.13	11.15	11.36	11.33	11.34	11.36	11.48	11.50	11.50	11.50	11.50
Ash	5.40	5.68	5.60	4.56	5.13	5.27	5.41	4.62	4.94	4.94	4.94	4.94
Crude protein	22.28	22.28	22.27	19.58	19.38	19.41	19.45	17.76	17.32	17.32	17.32	17.32
Crude fat	5.47	5.60	5.73	5.34	5.56	5.64	5.72	5.48	5.48	5.48	5.48	5.48
Crude fibre	4.02	4.03	4.05	4.09	3.85	3.87	3.87	3.90	3.88	3.88	3.88	3.88
FNE	51.71	51.26	50.82	55.05	54.74	54.46	54.17	56.75	56.86	56.86	56.86	56.86
Ca	0.98	1.00	1.00	0.65	0.90	0.88	0.87	0.7	0.82	0.82	0.82	0.82
P	0.81	0.80	0.80	0.66	0.74	0.74	0.75	0.65	0.66	0.66	0.66	0.66
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
ME. MJ/kg	12.79	12.61	12.43	12.96	13.03	12.93	12.82	13.10	13.09	13.09	13.09	13.09

The body-weight of broilers during the experiment is shown in Table 7. At the beginning of the test chickens had appropriate body weight for the provenance, the difference in weight between groups being statistically insignificant ($p>0.05$). The body weight of the chickens in the control group during the experiment remained within the limits prescribed by technological norms.

At the end of the first phase of fattening, statistically significant differences ($p<0.01$) were noticed between the control group and the three experimental groups. In the second and third phases these differences remained, but were not statistically significant ($p>0.05$).

Table 7. Broilers' body mass during the experiment (g)

Day of experiment	Group			
	Control	Experimental-I	Experimental-II	Experimental-III
01	44.95 ± 3.80	44.95 ± 3.84	44.94 ± 3.56	44.92 ± 4.26
21	837.60 ± 84.21 ^{A}	806.80 ± 76.24	784.00 ± 66.90	750.20 ± 80.82 ^{A}
35	1946.00 ± 226.69	1932.00 ± 183.64	1842.00 ± 183.67	1810.40 ± 213.68
42	2460.40 ± 347.12	2489.60 ± 279.05	2397.20 ± 301.57	2324.00 ± 275.98

[1] Value expressed in grams as $X \pm S_d$

Small letters^{a,b,c,d,e,f} $p < 0,05$; Capital letters^{A,B,C,D,E,F} $p< 0,01$

Statistical significances between groups are given per days (rows in tables)

The average daily weight gain of broilers in each phase of the research is shown in Table 8. In the control group it remained within the anticipated technological norms for this provenance throughout the experiment. However, broiler chickens in the control and experimental groups reached statistically different weight gain. In the first phase of the research a significant difference ($p<0.05$) was observed between the first and the third experimental group, and between the control and the third experimental group ($p<0.01$). However, in the second and third stages of the research, and in the entire essay as a whole the differences in weight gain between the experimental groups were not statistically significant.

Table 8. Weight gain[1] during the experiment (g)

Phase of experiment	Experimental group			
	Control	Experimental-I	Experimental--II	Experimental--III
01-21	37.75 ± 3.84 ^{A}	36.28 ± 3.47 ^{a}	35.19 ± 3.03	33.58 ± 3.67 ^{aA}
22-35	79.17 ± 10.32	80.37 ± 8.05	75.57 ± 8.81	75.73 ± 9.72
36-42	73.49 ± 19.90	79.66 ± 14.89	79.31 ± 18.28	73.37 ± 10.40
01-42	57.51 ± 8.19	58.21 ± 6.56	56.01 ± 7.11	54.26 ± 6.49

[1] Value expressed in grams as $X \pm S_d$
Small letters^{a,b,c,d,e,f} $p<0.05$; Capital letters^{A,B,C,D,E,F} $p<0.01$

The results obtained by chemical analysis showed that both worm meal and fresh red earthworms *Lumbricus rubellus* may be used for broiler feed. Results of the analysis of fresh earthworms showed that they are characterized by high water content (84.76%) and small amounts of other nutrients. The crude protein content in powder obtained from dried worms was 41.42%, which is higher than in sunflower meal (37.78%), but lower than in fish meal (67.39%). The protein contents in this research were lower in comparison to literature data, which range from 54.6 to 62% *(Cilevski, 1994; Zhen-Jun, 1995)*. Similarly, the examined content of amino acids was slightly lower compared to previous data, but still satisfying. The content of lysine and methionine in fresh worms ranged from 0.51 to 0.46 % and 0.14 to 0.13 % VSM and 7.40-7.90%, and 2.10 to 2.20 % per unit of protein.

In dehydrated worm meal lysine and methionine is about 3.33 and 0.96% VSM, or about 8.03 and the 2.32% per unit of protein. The data obtained on the absolute content of amino acids were significantly lower than those stated by *Nadaždin et al. (1988)* and *Cilevski (1994)*, but comparable if the content of amino acids per unit of protein is the criterion. Given the obtained results it can be concluded that worm meal is a satisfactory source of proteins and essential amino acids. On the other hand, fat and carbohydrate contents were higher in comparison

to literature data. According to *Dynes (2003)*, worm meal with highest fat contents originates from farms with low levels of subjective mobility of worms and low consumption of food. *Cilevski (1994)* argues that because of the relatively high percentage of fat in comparison to other nutrients, red earthworms are a rich source of energy if counted in dry matter. Ash, Ca and P contents corresponded to previously published data *(Reinecke and Alberts, 1987)*, but were slightly lower than in fish meal.

Chemical analysis of fatty-acid composition of red earthworm meal did not detect myristoleic and myristic fatty acids. The content of palmitic acid in worm meal was much higher than stated by *Daynes (2003)*, whilst the contents of stearic and linoleic acid only slightly higher. On the other hand, oleic acid was lower in worm meal, ranging from 12.14 to 13.14%; in comparison, fish meal contains 21.7-22.7%. The content of linoleic acid was significantly higher (4.77-4.91%) than content in fish meal (1.15 to 1.50%).

The analysis of heavy metals in the earthworm meal did not detect lead, cadmium and mercury, and the amount of arsenic were within acceptable limits *(Pravilnik Sl SFRJ 2/90 and 27/90)*.

Microbiological analysis showed that the earthworms and worm meal met the safety criteria prescribed *(Pravilnik, 4/2010)*.

The results of the chemical composition and hygienic quality analyses lead to the conclusion that dehydrated worm meal is a high-quality feed that may be successfully used for broiler chickens.

Feed mixtures used for broiler chickens in this research were composed of nutrients with quantitative participation common in our conditions.

The results of the chemical analysis of the broiler feed mixtures used in the control group showed that they met the technological norms and legislation *(Pravilnik, 4/2010)*, that is that the nutrient content in the mixtures fit the needs of broilers at different stages of fattening *(NRC, 1994)*. Feed for broiler chickens in experimental groups in which fish meal was replaced by worm meal did not differ from the control mixture. In chicken feed for O-III groups, protein contents were lower as the mixtures were made without the participation of feed of animal origin, but the chickens were allowed to consume fresh worms from day 1 ad libitum.
The results indicate that the chemical composition of complete feed mixtures for broilers in experimental groups fully met the needs of chickens and that corresponded to the requirements set in the experiment.

In comparison to the assessment of the nutritional value of feed based on chemical composition, feeding may provide much more complete information. In this case, one of the best indicators of the feed quality may be provided by production performance.

At the beginning of the experiment the body weight of broilers in all groups was uniform (44.9 ± 3.86 g), and differences between groups insignificant. At all stages the body weight of control broilers remained within the limits for the provenance *(Euribird, 1989)*. At the end of the first phase of fattening significantly lower body weight ($p<0.05$) was detected in the third experimental group compared to the control. At the end of the second and third phases of the research and on the whole, there were no major differences ($p>0.05$) in the body weight of broilers fed diets containing fish meal and that in which it was substituted with worm meal. These results are consistent with the statement made by *Cariage (1983)*, who assessed the nutritional value of earthworms and found no significant difference in body weight of broilers of the control and experimental groups. However, *Das andDash (1990)* reported that the average body weight of broilers fed worm meal was higher than in the control group.

Although body weight is a good indicator of feed quality, it is considered that the daily weight gain is a more reliable indicator. On analyzing the results it can be concluded that the daily weight gains of broilers in the control group during the experiment were within the anticipated technological norms for the provenance. In the first stage, chickens in O-III group fed without nutrients of animal origin but with the addition of fresh worms had significantly ($p<0.01$) lower weight gain than both the control and the OI group ($p<0.05$). In the second and the third phase of the research as well as in the experiment as a whole the differences between the groups were not significant ($p>0.05$). Virtually identical data on a slightly lower weight gain in the first phase of fattening and the improvement in the second phase was reported by *Rodriguez et al. (1995)* and *Cilevski (1994)*. Good results in growth, affected by the use of earthworm meal in poultry feed were stated in the works of *Zhen Jun (1995)* and *Das and Dash (1990)*.

In general, it can be concluded that worm meal in broiler feed may provide the same weight gain as fish meal does in accordance with the recommendations of the *NRC (1994)*. In the experiment no effects of anti-nutritive factors were observed in the third experimental group, which is consistent with the data provided by *Cilevski (1994)* and *Zhen Jun (1995)*.

Having considered the production results in the experiment as a whole, it can be concluded that there were no significant differences in production results between the groups; weight gain and body mass were within the limits of the given provenance, which suggests that worm meal and fresh earthworms may serve as a good source of proteins in poultry feed. It should be pointed out that worms and worm meal can be produced at low cost and without expensive technology. These findings confirm the results of other authors *(Cariaga, 1983; Dioson, 1985, Das and Dash, 1990; Zhen-Jun, 1995)*.

Conclusion

According to the results obtained it can be concluded that fresh worms contain large quantities of water (84.76%) and a small amount of other nutrients. Dehydrated worm meal is characterized by high protein content - 41.42% (DM) and sufficient amounts of lysine, methionine and cystine. The nutritional value of worm meal is somewhat lower than the one of fish meal. Fat content in dehydrated worm meal (9.20% DM) is slightly higher than in fish meal and its fatty acid composition is favorable. According to the results of heavy metal detection and bacteriological analysis, it can be concluded that dehydrated worm meal and fresh earthworms (*Lumbricus rubellus*) are hygienic and safe feed which can be used in broilers without adverse health effects.

Given the production results of the experiment as a whole, it can be concluded that broilers fed diets with the isoprotein substitution of fish meal by worm meal achieved satisfactory production results. Broilers readily consumed fresh biomass and having in mind that its amino acid content is satisfactory, there is a reasonable possibility of using fresh worms in broiler reared under conditions of organic production.

Prerada stajnjaka kalifornijskom glistom *(Lumbricus Rubellus)* i mogućnost upotrebe biomase u ishrani živine

Lj. Janković, B. Radenković-Damnjanović, M. Vučinić, Š. Pintarič

Rezime

Cilj rada je bio da se ispita hranljiva vrednost biomase glista *(Lumbricus rubellus)* i brašna od glista, kao i uticaj ishrane smešama u kojima je izvršena zamena ribljeg brašna brašnom od glista i svežim glistama, na zdravstveno stanje i proizvodne rezultate brojlera. Rezultati hemijskih analiza su pokazali da brašno od glista sadrži 41.42% proteina (SM) i zadovoljavajuću količinu amino kiselina.

Ogled izveden na 100 brojlera *Hybro* provenijence, podeljenih u četiri grupe trajao je 42 dana. Brojleri kontrolne grupe su dobijali hranu standardnog sirovinskog i hemijskog sastava, dok je u smešama za ishranu prve i druge eksperimentalne grupe izvršena zamena ribljeg brašna u količini od 50, odnosno 100%, brašnom od glista. Treća eksperimentalna grupa hranjena je smešama iz kojih je potpuno isključeno riblje brašno uz dodatak svežih glista *ad libitum.* Na kraju prve faze tova treća ogledna grupa postigla je statistički značajno nižu telesnu

masu ($p<0.05$) u odnosu na kontrolnu grupu. Na kraju druge i treće faze tova, kao i za ceo ogled zbirno, nije bilo velikih numeričkih razlika između telesne mase brojlera hranjenih smešama sa ribljim brašnom i smešama u kojima je riblje brašno supstituisano brašnom glista, tako da između grupa nije utvrđena statistički značajna razlika ($p>0.05$) u odnosu na kontrolnu grupu. Razlike u dnevnom prirastu između grupa nisu bile značajne ($p>0.05$).

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IMPACT OF NUTRITION ON GROWTH DYNAMICS AND QUALITY OF BROILER CARCASS OF DIFFERENT GENOTYPES

N. Tolimir[1], L. Perić[2], N. Milošević[2], M. Đukić – Stojčić[2], M. Maslovarić[1]

[1]Institute of Science Application in Agriculture,' Bulevar Despota Stefana 68b, 11000, Belgrade, Republic of Serbia
[2]Faculty of Agriculture Novi Sad, Trg Dositeja Obradovića 8, 21000, Novi Sad, Republic of Serbia
Corresponding author: e-mail: ntolimir@ipn.co.rs
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Abstract: The objective of the present study was to investigate the effect of feeding programs with different frequency of changes in starter, grower and finisher feed on the dynamics of growth and carcass quality of broilers of different genotypes. The experiment was conducted on 1200 Cobb 500 and Ross 308 chickens. In the experimental period of 42 days standard technology was used. The difference between the groups (treatments) was in the type of mixture used in the starter period, according to the following phase feeding program: T1 (control group) – broilers were fed mixture containing 23% protein from 1 to 21 days of age; T2 - nutrition from 1 to 14 days of age with a mixture containing 23% and from 14 to 21 days of age mixture containing 20% protein, T3 – nutrition, mixture containing 23% of protein was used from 1 to 7 days and from 7 to 21 days 20% protein mixture; T4 – nutrition, mixture containing 20% protein was used from 1 to 21 days. According to the results obtained, the growth dynamics of chickens was significantly under the influence of genotype and nutrition programs. When compared with the control T1 group, in experimental groups of chickens of both genotypes, at the level of the trial, significant differences were found in the average daily gain (ADG) level only between T1 and T4 groups, while the smallest difference was between the T1 and T2 groups. Share of breasts, drumsticks with thighs and abdominal fat in both hybrids was not under significant influence of studied feeding treatments. However, although the differences were not statistically significant, it was evident that T4 treatment recorded the lowest values for the share of breast and usually the highest values for abdominal fat, indicating a negative impact of feeding programs with the greatest reduction in protein. Based on the obtained results it can be concluded that the applied treatments with drastic dietary protein restriction in the starter period in both genotypes resulted in negative trends in the dynamics of growth and carcass quality. From the

perspective of the development of cost-effective nutrition program, the focus of further investigations should be on the T2 diet program.

Key words: broiler, genotype, phase feeding, breast, drumstick, abdominal fat

Introduction

The primary objectives of the intensive selection of broilers in recent decades have been the final body weight, i.e. growth rate, feed efficiency, vitality, forming of plumage, conformation and carcass quality of meat (*Milošević and Supić, 1995*). The expression of the genetic potential of broilers and maximum productivity is only possible under optimal conditions of growing and nutrition. There is difference between the genotypes in the needs for protein and other nutrients in the diet (*Cobb 500, 2007; Ross 308, 2007*). In broiler production, most often used programs are based on the recommendations contained in the NRC standards (*NRC, 1994*) or defined by the selection/breeding centres and manufacturers of certain line hybrids. However, in addition to the usual programs of broiler nutrition, an interest is noticed in developing new programs to satisfy the requirements of cost reduction in terms of nutrition, specific requirements of the market (production of special quality meat, reduction of fat in the carcass, etc.), of growth intensity, environmental requirements or other needs of modern broiler production. In the last decade, programs based on different frequencies of changes in certain mixtures, i.e. starter, grower and finishers in broiler nutrition, have been in the focus of the attention. The main objective of this phase feeding program is to determine the optimal proportion of time for feeding basic mixtures, in terms of production and slaughtering performance of broilers and production efficiency, but often environmental aspects of these programs is perceived, and this issue studied by a number of authors (*Watkins et al., 1993; Warren and Emmert, 2000; Pope and Emmert, 2001; Roush et al., 2004*). The economic impact of replacing the starter with grower mixture in the past was based on the difference in cost between the two compounds, with some studies indicating that mixtures with reduced protein do not affect body weight, but has a significant impact on the economic efficiency (*Warren and Emmert, 2000*). Research suggests that the proportion of time feeding starter, grower and finisher diets depends on the desired final weight at the end of the fattening (*Saleh et al., 1997a; Saleh et al., 1997b).*

The objective of the present study was to investigate the effect of feeding programs with different frequency of changes in starter, grower and finisher feed on the dynamics of growth and carcass quality of broilers of different genotypes.

Material and Methods

The experiment was conducted at the experimental farm of the Faculty of Agriculture in Novi Sad. The study was carried out on 1200 individually tagged chickens of Ross 308 and Cobb 500 hybrids. The chickens were housed in 16 boxes with 75 chickens in each box. There were four groups within each genotype - treatment (150 chicks per group), with two replicates for each treatment.

The standard fattening technology was applied, for a period of 42 days. Feeding chickens was *ad libitum***.** The difference between the groups (treatments) was in the type of mixture used in the starter period, according to the following phase feeding program: T1 (control group) – broilers were fed mixture containing 23% protein from 1 to 21 days of age; T2 - nutrition from 1 to 14 days of age with a mixture containing 23% and from 14 to 21 days of age mixture containing 20% protein, T3 – nutrition, mixture containing 23% of protein was used from 1 to 7 days and from 7 to 21 days 20% protein mixture; T4 – nutrition, mixture containing 20% protein was used from 1 to 21 days of age.

Control measurements were performed on the first day and in weekly intervals from 1 to 6 weeks. Based on data on body weight of chickens in weekly intervals weekly gain was calculated, i.e. average daily gain by weeks, as well as daily gain for the entire experimental period. In order to investigate the slaughter traits in both genotypes, at the age of 42 days, 8 chickens per both sexes and for each diet treatment were set aside. Chickens used in the study of slaughter traits were processed/cut according to the *Regulation on the quality of poultry meat (1981).* Carcasses and individual carcass parts were weighed using a technical scale, precision of ± 0.1 g. To determine the yield of certain carcass parts, processed carcasses were cut according to the above mentioned Regulation. During further processing of carcasses the abdominal fat was separated. In calculating the share of certain carcass parts, they were put in the relation to the pre-slaughter body weight of chickens.

In data analysis computer program Statistika, Anova Manova and LSD post-hoc test were used.

Results and Discussion

Based on the results obtained it was established that the applied programs of phase broiler nutrition had certain impact on the dynamics of growth and carcass quality of broilers of different genotypes.

Table 1 presents the evaluation of the significance of differences established in the impact of the main factors on average daily gain of Cobb 500 and Ross 308 hybrids per week. At the level of the experiment, it was established that the hybrid had a statistically significant effect in the first, fourth and fifth week, and the food as a factor of variability had a statistically significant effect on body weight in all experimental weeks.

In both studied genotypes, when comparing the control group T1 with the experimental groups, significant and at the same time the highest difference in average daily gain by week and at the level of whole trial (Table 2) was determined between T1 and T4 groups in all weeks except week 4. By comparing experimental groups, in chickens of both genotypes it was observed that the experimental group with the greatest reduction in protein (T4) recorded significantly lower growth in comparison with T2 and T3 group, almost in all weeks and at the level of whole trial. Delayed growth of chickens in this group during the starter period, but also in the second fattening phase, is caused by the drastic reduction of the protein, since the chickens consumed grower mixture from the day 1. The results are consistent with studies in which the chickens exposed to drastic reduction of protein during the early period lagged behind in their growth, and at a later stage failed to compensate for the loss *Watkins et al. (1993).*

The results obtained in a certain way can be related to research by *Jianlin et al. (2004)* who have studied eight feeding programs during the starter period in broiler chickens, while the control group consumed feed with 22.48% of crude protein, and in each subsequent group protein content was reduced, and the lowest was 16.61%. In this study, body weight of the chickens in experimental group had lower values compared to the control group, and with the content being more reduced also the body weight was lower.

Table 1. Daily weight gain of chickens (g) of Cobb 500 and Ross 308 hybrids per week of age

Week	Variation measure	Average daily gain (g)										
		Cobb 500				Ross 308				Factor		
		T1	T2	T3	T4	T1	T2	T3	T4	Hybrid	feed	HxF
1.	$\bar{x}$	15,05^{a}	15,62^{a}	15,11^{a}	11,68^{b}	17,35^{a}	17,52^{a}	16,94^{a}	12,59^{b}	**	**	*
	Sd	2,74	2,41	2,66	1,89	2,50	2,56	2,74	2,25			
2.	$\bar{x}$	27,02^{a}	26,60^{a}	24,76^{b}	20,44^{c}	25,33^{a}	26,53^{a}	22,77^{b}	22,50^{b}	NS	**	**
	Sd	6,76	5,34	4,08	4,31	5,59	6,01	6,20	4,76			
3.	$\bar{x}$	44^{a}	41^{b}	42^{ab}	38^{c}	44^{a}	42^{a}	44^{a}	39^{b}	NS	**	NS
	Sd	11,43	8,83	8,85	9,19	8,40	9,74	9,40	10,38			
4.	$\bar{x}$	61^{b}	66^{a}	67^{a}	60^{b}	69^{a}	71^{a}	72^{a}	68^{a}	**	**	NS
	Sd	13,94	10,15	11,19	9,48	16,75	18,40	17,82	15,29			
5.	$\bar{x}$	85^{a}	76^{b}	77^{b}	74^{b}	65^{a}	59^{b}	63^{a}	52^{c}	**	**	**
	Sd	12,76	13,17	10,65	13,87	14,59	13,88	15,72	11,68			
6.	$\bar{x}$	88^{a}	86^{a}	83^{b}	83^{b}	86^{a}	88^{a}	81^{b}	81^{b}	NS	**	NS
	Sd	14,11	17,65	14,20	14,77	13,95	14,64	16,53	18,56			

a-b Values within rows according to sex that don't have the same letter in superscript are statistically significantly different (P <0.05)

* statistical significance (P<0.05)

** statistical significance (P<0.01)

NS no statistical significance

Investigation of the effect of a diet containing different levels of protein in multiphase feeding programs was performed also by *Dozier et al. (2006),* and the results indicated that the higher content of protein and nutrients in the diets resulted in greater body weight.

Table 2 presents the assessment of significance of differences in the influence of major factors on the average daily gain of Cobb 500 and Ross 308 broilers at the level of the whole trial.

Table 2. Daily gain of chickens (g) Cobb 500 and Ross 308

Period	Variation	Average daily gain (g)										
		Cobb 500				Ross 308				Factor		
		T1	T2	T3	T4	T1	T2	T3	T4	Hybrid	Feed	HxF
1- 42	$\bar{x}$	53,37[a]	51,85[a]	51,56[a]	47,89[b]	50,56 [a]	50,67 [a]	49,96 [a]	44,10 [b]	NS	**	NS
	Sd	6,22	5,81	5,00	5,58	7,24	6,27	6,83	6,74			

a-b Values within rows according to sex that don't have the same letter in superscript are statistically significantly different (P <0.05)
** statistical significance (P<0.01)
NS no statistical significance

In hybrids Cobb 500 and Ross 308, the smallest, insignificant difference in average daily gain at the level of whole trial, was determined by comparing T1 with the group that consumed the starter mixture for a period of two weeks (T2). This result imposes the question of whether the chickens in the starter period needed starter feed longer than 14 days and indicates the need for further studies. The results are consistent with research by *Saleh et al. (1997a)* who evaluated the proportion of time in feeding chickens starter, grower and finisher diets in fattening to 42 days. This author has pointed out the importance of the target final weight in planning nutrition programs in terms of duration of particular phases of food, and made recommendations for duration of use of certain mixtures depending on whether fattening is up to 1kg, 2kg or 3kg (*Saleh et al., 1996; Saleh et al., 1997b*).

Table 3 shows the absolute and relative yields of breasts, drumsticks and thighs, i.e. the most valuable parts of the carcass, for the Cobb 500 and Ross 308 hybrids.

Table 3. The yield and the share of breasts and drumsticks with thighs in Cobb 500 and Ross 308 chickens

Trait	Variation measure	Cobb 500										
		Male chickens				Female chickens				Factor		
		T1	T2	T3	T4	T1	T2	T3	T4	Sex	Feed	SxF
Breast yield (g)	$\bar{x}$	602,05	606,00	634,02	583,33	527,83	510,80	523,29	506,33	**	NS	NS
	Sd	65,25	46,68	45,39	94,32	53,18	78,58	65,46	63,72			
Breast share (%)	$\bar{x}$	24,90	25,71	25,61	24,46	25,67	25,04	25,18	24,96	NS	NS	NS
	Sd	2,59	1,87	1,53	1,50	0,98	1,74	1,78	1,14			
Yield of drumsticks with thighs (g)	$\bar{x}$	514,17	496,84	532,17	506,00	426,00	431,01	427,57	415,50	**	NS	NS
	Sd	77,48	33,98	43,63	76,78	42,05	35,82	36,18	31,61			
Share of drumsticks with thighs (%)	$\bar{x}$	21,32	21,06	21,46	21,23	20,73	20,96	20,78	20,59	NS	NS	NS
	Sd	1,13	0,75	0,72	0,95	0,90	0,50	1,10	1,13			
Trait	Variation measure	Ross 308										
		Male chickens				Female chickens				Factor		
		T1	T2	T3	T4	T1	T2	T3	T4	Sex	Feed	SxF
Breast yield (g)	$\bar{x}$	532,40	547,80	533,30	525,00	420,00	460,00	457,40	431,80	**	NS	NS
	Sd	43,26	19,08	44,58	35,54	42,12	15,65	24,50	28,24			
Breast share (%)	$\bar{x}$	24,21	24,70	24,25	23,70	23,96	23,94	23,40	23,31	NS	NS	NS
	Sd	1,47	1,18	2,18	1,29	1,06	0,85	1,05	1,23			
Yield of drumsticks with thighs (g)	$\bar{x}$	466,30	468,80	466,10	472,00	391,30	388,40	397,20	381,40	**	NS	NS
	Sd	15,47	14,41	11,37	24,75	36,33	12,24	24,75	34,36			
Share of drumsticks with thighs (%)	$\bar{x}$	21,24	21,12	21,17	21,35	20,26	20,21	20,31	20,61	**	NS	NS
	Sd	0,90	0,68	0,33	1,04	0,62	0,70	0,97	1,17			

** statistical significance (P<0.01)
NS no statistical significance

In hybrids Cobb 500 and Ross 308, impact assessments of dietary factors showed statistical significances in yields of breasts and drumsticks with thighs, suggesting that different programs of phase feeding had no effect on these carcass traits. However, for both investigated hybrids it could be observed that the treatment T4 recorded the lowest values for the share of the breast, which may be related to the influence of diet. In dealing with similar issues *Watkins et al. (1993)*

have found the largest share of chicken breast in the groups of chickens that consumed starter mixture during the starter period of 21 days, and the lowest proportion in the group that consumed the starter only the first 7 days, which is in accordance with the results obtained. The results of this study are also consistent with research by *Saleh et al. (1997a) and, Saleh et al. (1997b)*, who studied different representation of starter mixture during the first 21 days (7, 14 or 21 days) and found that the proportion of breast muscle was proportional to time of feeding the starter mixture.

The yield and the share of abdominal fat of Cobb 500 and Ross 308 chickens, within each gender and studied treatment are shown in Table 4.

Table 4. Yield and share of abdominal fat of Cobb 500 and Ross 308 chickens

Trait	Variation measure	Yield and share of abdominal fat										
		Cobb 500										
		Male chickens				Female chickens				Factor		
		T1	T2	T3	T4	T1	T2	T3	T4	Sex	Feed	Interact.
Yield of abdominal fat (g)	$\bar{x}$	21,50	22,17	22,50	24,67	19,50	20,83	20,67	22,67	NS	NS	NS
	Sd	1,64	3,97	4,85	3,94	5,01	6,11	3,08	5,32			
Share of abdominal fat (%)	$\bar{x}$	0,91	0,94	0,91	1,06	0,95	1,01	1,02	1,12	NS	NS	NS
	Sd	0,16	0,12	0,21	0,22	0,24	0,22	1,06	0,23			
		Ross 308										
		Male chickens				Female chickens						
Yield of abdominal fat (g)	$\bar{x}$	23.10	23,70	24,30	25,10	21,40	21,30	22,90	22,80	NS	NS	NS
	Sd	4,01	5,66	2,79	4,09	3,50	1,42	4,80	3,52			
Share of abdominal fat (%)	$\bar{x}$	1,05	1,07	1,10	1,14	1,11	1,11	1,17	1,24	NS	NS	NS
	Sd	0,17	0,26	0,12	0,18	0,15	0,07	0,23	0,22			

** statistical significance (P<0.01)
NS no statistical significance

In the present study, slightly smaller share of abdominal fat in chickens of Cobb 500 was established and the values obtained for both hybrids were approximate to those in the study by *Petričević et al. (2011).* Analysing the impact of nutrition, in both sexes of chickens Cobb 500 and Ross 308, no statistical significance was determined by comparing the differences in yield and share of abdominal fat. However, although the differences were not statistically significant, it was evident that T4 treatment generally had the highest values for abdominal fat

in both sexes of studied genotypes, indicating a negative impact of feeding programs with the greatest reduction in protein. The results obtained can be associated with the research which has shown that higher protein content in diets results in lower-abdominal fat content *(Fisher, 1984; Smith et al., 1998).*

Conclusion

Feeding programs included phase diet/nutrition, with varying frequency of change of the starter, grower and finisher mixtures used for nutrition of Cobb 500 and Ross 308 chickens, in order to optimize production in economic terms. In Cobb 500 and Ross 308 hybrids, drastic protein reduction in the T4 diet program had a negative impact on the dynamics of growth, i.e. significant decline in average daily gain in almost all weeks, as well as at the level of the whole trial. In both genotypes, the smallest, insignificant difference in average daily gain at the level of whole trial was determined by comparison with a control group that consumed the starter mixture for a period of two weeks, which suggests the need for further testing of this diet program. The applied phase feeding programs did not result in significant effects on carcass quality in terms of yield of breasts, thighs and drumsticks with abdominal fat, but treatment with a drastic protein reduction resulted in the lowest value for share of breast and the highest values for abdominal fat, suggesting a negative effect of this feeding program.

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Uticaj programa ishrane na dinamiku porasta i kvalitet trupa brojlera različitih genotipova

N. Tolimir, L. Perić, N. Milošević, M. Đukić – Stojčić, M. Maslovarić

Rezime

Cilj rada je ispitivanje uticaja programa ishrane sa različitom frekvencijom promene starter, grover i finišer smeša za ishranu na dinamiku porasta i kvalitet trupa brojlera različitih genotipova. Ispitivanje je izvedeno na 1200 pilića provenijenci Cobb 500 i Ross 308. U oglednom periodu od 42 dana primenjena je

standardna tehnologija. Razlika između grupa (tretmana) bila je u tipu smeša u starter periodu, prema sledećem programu fazne ishrane: T1 (kontrolna grupa) - ishrana smešom sa 23% proteina u trajanju od 1. do 21. dana; T2 - ishrana od 1. do 14. dana smešom sa 23% i od 14. do 21. dana sa 20% proteina; T3 - ishrana od 1. do 7. dana smešom sa 23% i od 7. do 21. dana sa 20% proteina; T4 - ishrana smešom sa 20% proteina u trajanju od 1. do 21. dana. Prema dobijenim rezultatima, dinamika porasta pilića bila je pod signifikantnim uticajem genotipa i programa ishrane. Pri poređenju kontrolne T1 sa oglednim grupama, kod pilića oba genotipa, za prosečan dnevni prirast na nivou ogleda utvrđene su signifikantne razlike samo između T1 i T4 grupe, dok je najmanja razlika bila između T1 i T2 grupe. Udeo grudi, bataka sa karabatakom i abdominalne masti kod obe provenijence nije bio pod signifikantnim uticajem ispitivanih tretmana ishrane. Međutim, iako razlike nisu imale statističku značajnost, uočava se da je T4 tretman ostvario najniže vrednosti za udeo grudi i uglavnom najviše vrednosti za abdomnalnu mast, što ukazuje na negativan uticaj programa ishrane sa najvećom redukcijom proteina. Na osnovu dobijenih rezultata može se zaključiti da su primenjeni tretmani ishrane sa drastičnom restrikcijom proteina u starter periodu kod oba genotipa rezultirali negativnim tendencijama na dinamiku porasta i kvalitet trupa. Sa aspekta razvoja ekonomičnijih programa ishrane, težište daljih ispitivanja trebalo bi da bude na T2 programu ishrane.

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THE EFFECT OF COCONUT WASTE WITH TOFU WASTE MIXTURE FERMENTED BY *ASPERGILLUS NIGER* ON BROILER PERFORMANCE

F. R.Wolayan[1], A. Rochana T.[2], I. Setiawan[2], Y. A. Hidayati[2]

[1]Faculty of Animal Husbandry Sam Ratulangi University, Manado, Indonesia
[2]Faculty of Animal Husbandry, Universitas Padjadjaran, Bandung, Indonesia
Corresponding author: rinayw@yahoo.com
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Abstract: This study aimed to determine the effect of the mixture of coconut waste and tofu fermented with *Aspergillus niger* in ration on feed intake, weight gain and feed conversion of broiler chickens. This research used Completely Randomized Design (CRD) consisted of 6 treatments, (the usage rate of fermentation products of 0, 5, 10, 15, 20 and 25 per cent) in the ration, and each treatment was repeated four times. Statistical tests performed by analysis of variance and differences between treatments effects were examined using Duncan's multiple range test. Results indicated that using up to level 20% in ration supported the performance of broiler chickens (feed consumption 2511.60 g; the final weight 1585.36 g, carcass percentage 68.13% and feed conversion 1.58kg/kg).

Key words: coconut waste, tofu waste, *Aspergillus niger*, fermentation, broiler

Introduction

Utilization of waste as an alternative to animal feed is necessary because almost the constituent ingredients of feed rations are also human food. To avoid that, it is necessary to look for other feed ingredients that do not compete with human needs. Agricultural processing wastes, such as waste oil dregs and waste processing waste (VCO), can be utilized as raw material for animal feed rations constituent. The problem of using processing waste is the poor nutrition quality. The fermentation process can be applied to improve the quality of the waste using microbes.

Aspergillus niger is a type of mold that is used commercially in improving the quality of agricultural processing wastes, because of the easy handling, its ability to grow quickly and it is not harmful because it does not produce mycotoxins. This fungus can produce enzymes such as a-amylase, amylase,

cellulase, gluco-amylase, catalase, pectinase, lipase, and galactosidase (*Ratledge, 1994*).

The use of *Aspergillus niger* fermentation product on agricultural wastes for animal feed has been tested by *Abun (2003*), using the fungus *Aspergillus niger* fermentation products as broiler feed ingredients, that can improved the broiler chickens performance. Therefore, it is interested to do disclosure of the extent the performance of broiler chickens using a mixture of coconut pulp (VCO waste).

Material and Methods

Materials

Preparation of fermentation products :

- Coconut waste (VCO waste), from the coconut oil processing home industry (VCO) North Minahasa, North Sulawesi, Indonesia.
- Tofu waste, obtained from the Industri Pengolahan Tahu Cikuda, Sumedang; West Java, Indonesia
- Fungus *Aspergillus niger*, obtained from the Laboratory of Biological Sciences, Institut Teknologi Bandung; Indonesia
- Rice, fresh sprouts, gelatin, sugar, obtained from Pasar Suci, Bandung, Indonesia.

Animal experiment

Animal experiments used 120 Cobb strain (straight run) day-old chickens broiler from PT. Missouri Bandung, Indonesia for 5 weeks (35 days).

Rations

Feed ingredients used in the experiment consisted of a compiler ration of yellow corn, fish meal, soybean meal, palm oil, DCP, Top Mix, CaCO3, and the mixture of coconut pulp (VCO waste) and fermented tofu products. Feed materials were obtained from PT. Missouri Bandung.

Variables studied

Feed intake, body weight and feed conversion.

Research Design

This research used Completely Randomized Design *(Steel and Torrie, 1991)*, with 6 treatments and each treatment was repeated 4 times. (there are 24 experimental units using 120 chickens, so each unit were placed 5 chickens).

Treatment rations were given as follows:
R0 = Ration containing 0% fermentation products (CAKTAF) as control
R1 = Ration containing 5% fermentation products (CAKTAF)
R2 = Ration containing 10% fermented products (CAKTAF)
R3 = Ration containing 15% fermented products (CAKTAF)
R4 = Ration containing 20% fermented products (CAKTAF)
R5 = Ration containing 25% fermented products (CAKTAF)

Note: CAKTAF is Coconut Waste Mixture with Tofu Waste Fermented.

In Table 1, the composition of rations for each treatment is presented.

Table 1. Composition of Rations Research (%)

Feed Contents	Treatments					
	R0	R1	R2	R3	R4	R5
Corn meal	56.44	52.44	48.44	44.44	40.44	36.44
Soybean meal	29	28	27	26	25	24
Fish meal	9	9	9	9	9	9
Coconut oil	2.5	2.5	2.5	2.5	2.5	2.5
Methionine	0.16	0.16	0.16	0.16	0.16	0.16
Top mix	0.5	0.5	0.5	0.5	0.5	0.5
CaCO3	0.9	0.9	0.9	0.9	0.9	0.9
Dicalcium phosphate	1.5	1.5	1.5	1.5	1.5	1.5
CAKTAF	0	5	10	15	20	25
Total	100	100	100	100	100	100

Note: Compiled by standard requirement by Lesson and Summer (2001)

From Table 1, the compositions are differs only in corn meal, soybean meal and CAKTAF. And in Table 2, the substances contents of feed experiments.

Table 2. Substances Content of Feed contents

Feed Contents	Treatments					
	R0	R1	R2	R3	R4	R5
Protein (%)	22.56	22.49	22.43	22.37	22.31	22.25
EM Kcal/kg)	3023.97	3025.61	3027.25	3028.89	3030.53	3032.17
Fat (%)	6.45	6.41	6.36	6.31	6.28	6.22
Crude fiber (%)	3.40	4.44	4.32	4.40	4.41	4.41
Lysin (%)	1.33	1.33	1.33	1.33	1.32	1.32
Methionine (%)	0.60	0.59	0.59	0.59	0.59	0.59
Met + Cyst (%)	0.93	0.93	0.92	0.91	0.91	0.90
Ca (%)	1.08	1.09	1.09	1.09	1.09	1.09
P-non phytate (%)	0.52	0.52	0.52	0.51	0.51	0.51

Results and discussion

The average of feed consumption, body weight and feed conversion were obtained from the results of the study are shown in Table 3.

Table 3. The average of feed consumption, body weight and feed conversion

Component	Treatments					
	RO	R1	R2	R3	R4	R5
Consumption (g)	2590.67	2585.30	2556.04	2549.22	2511.60	2329.78
Body Weight (g)	1650.32	1633.85	1618.21	1608.84	1585.36	1411.07
Feed Conversion (Kcal/kg)	70.79	69.49	69.78	69.78	68.13	67.20

The effects on Feed Consumption

From the observations, the average of feed consumption during the study (5 weeks) ranged from 2329.78 g to 2590.67 g, as shown in Table 3. The effect of feed consumption were analyzed by variance test. Results of the variance test showed that CAKTAF treatment levels used in the ration has significantly effects ($p < 0.05$) on feed consumption of broiler chickens. To find out how much the differences between the treatment on feed consumption were used Duncan's multiple range test, and the results are presented in Table 4.

Table 4. Duncan's Multiple Range on Feed Consumption Test During Research

Treatments	Average Feed Consumption (g)	Significancy ($p<0.05$)
R0	2590.67	a
R1	2585.30	a
R2	2556.04	a
R3	2549.22	a
R4	2511.60	a
R5	2329.70	b

Note : The same letter in the significancy column showed no significant

By Duncan test, results showed that the usage rates of CAKTAF (5%, 10%, 15% and 20%) in the rations raised the chicken feed consumption; which were not significantly different ($p < 0.05$), but the using of 25% CAKTAF has

significant effect (p <0.05), which were lower in comparison with other treatments (without CAKTAF- 0%; 5%, 10%, 15% and 20% CAKTAF). This facts illustrated that the addition of CAKTAF up to 20% level (R0 till R4) in ration, on feed intake were able to well respond.

Feed consumption of broilers was influenced by the palatable of feed ingredients making up the ration. The low of 25% CAKTAF (R5) consumption was caused by the expandable nature of fermentation products used in the ration. The higher it expands, it will accelerate the onset of strain that causes chicken gizzards stopped in feed consumption. This is consistent with *Amrullah, (2004);* who stated that feed consumption was also determined by whether or not the cache is full, so effort to increase the feed intake; according to the energy needs per day is limited. Beside that, feed intake is influenced by the palatable of feed ingredients making up the ration.

Ration palatable are depends on smell, taste, color and texture of the feed material (*Parrakasi, 1995*). CAKTAF usage at a rate of 25% in the ration, significantly influenced (p <0.05) the decrease in feed intake compared to the control ration treatment (R0). This illustrated that the diet containing CAKTAF at a rate of 25% raises the texture or expands the ration so that the palable ration decreased.

The effects on BodyWeight

The results of the average value chicken weight gain during the study (5 weeks) ranged from 1441.07 g until 1650.32 g, as seen in Table 3. Then the differences of the treatment on weight gain were analyzed. Results show that the ration which contents of fermentation products showed significance (P <0.05) on body weight of broiler chickens. And then, by Duncan multiple range test to find out the differences between treatments are shown in Table 5.

Table 5. Duncan's Multiple Range Test on Body Weight

Treatments	Average Body Weight (g)	Significancy (p<0.05)
R0	1650.32	a
R1	1633.85	a
R2	1618.21	a
R3	1608.84	a
R4	1585.36	a
R5	1441.07	b

Note: The same letter in the significancy column showed no significant difference

By Duncan tests, showed that the body weight of R1, R2, R3, R4 and R5 decreased compared to R0, but R1, R2, R3, and R4 has significancy ($p < 0.05$) than R5. It means that CAKTAF (5%, 10%, 15% and 20%) in the diet has no significant on weight gain of broilers ($p < 0.05$) compared to R0, but 25% CAKTAF in ration showed significant difference ($p < 0,05$); that decreased the weight gain compared to the ration 0% CAKTAF (control) and contained CAKTAF of 5%, 10%, 15%, and 20%. This illustrated that the addition until 20 CAKTAF in the ration, still useful on weight gain of broilers.

This results are supported to the improvement on nutritional value, especially on coconut and tofu dregs fermented mixture. In accordance with the facts found in the first and second phase of the research; that the protein content increased, decreased crude fiber and crude fat, and metabolizable energy value and protein digestibility increased compared with CAKTAF ration. The fermentation process by *Aspergillus niger* is able to break down the proteins into smaller compounds in the form of amino acids, was easily absorbed by broiler chickens.

The low weight gain of broilers that fed ration content 25% CAKTAF is caused by the reduced of feed intake. That is, in line with the opinion of *North and Bell (1990)*, who stated that the growth rate depends on the amount of feed intake, in addition to gender and age.

The effect on Feed Conversion

From the observations, the average of broiler feed conversion during the study (5 weeks) ranged from 1.6174 Kcal/kg to 1.5661 Kcal/kg, as shown in Table 3. The differences in the treatments, has effects on feed conversion analysis of variance. The variance analysis showed that the rations containing fermentation products was not significantly influenced ($p>0.05$) the value of broiler feed conversion. The feed conversion ratio was not different between treatments due to the balance between feed intake and body weight gain. It means that the chicken which consumed high diets would generate high weight gain, otherwise the chicken which consumed low diets would generate low weight gain, in accordance with the statement of Leeson and Summers (2001), the feed conversion ratio obtained from the comparison between feed intake and weight gain achieved during a certain time.

The average value of broiler feed conversion during the study ranged from 1.57 Kcal/kg to 1.62 Kcal/kg. This results is in line with the Cobb standard, that the value of feed conversion of Cobb strain broilers during 5 weeks maintenance ranged from 1.47 Kcal/kg to 1.61 Kcal/kg. High value of feed conversion showed that the sum of ration needed to increased the weight gain. So, the lower the value of the feed conversion, the better the ration quality. Thus the value of broiler feed

conversion results are in the normal range. A low value of feed conversion describes the efficiency of using ration to produce a good weight gain. It means that the quality of the ration in this study are good.

Conclusion

From the observation and analysis performed, showed that using a mixture of coconut pulp (VCO waste) and tofu fermented by *Aspergillus niger* up to 20% in broiler rations can support the performance of broiler chickens.

Uticaj smeše kokosovog i otpada tofua fermentisane pomoću *Aspergillus niger* na proizvodne rezultate brojlera

F. R.Wolayan, A. Rochana, I. Setiawan, Y. A. Hidayati

Rezime

Ova studija ima za cilj da utvrdi uticaj smeše kokosovog otpada i tofua fermentisane sa *Aspergillus niger* u obroku na unos hrane, prirast i konverziju hrane brojlerskih pilića. Ovo istraživanje koristi kompletno nasumični dizajn ogleda (CRD) koji se sastojao od 6 tretmana, (stopa upotrebe proizvoda fermentacije od 0, 5, 10, 15, 20 i 25 odsto) u obroku, a svaki tretman je ponovljen četiri puta. Statističko testiranje je obavljeno korišćenjem analize varijanse i razlike između tretmana efekata ispitane su korišćenjem Duncan-ovog testa višestrukog opsega. Rezultati ukazuju da korišćenje do 20% u obroku podržava proizvodne performanse brojlera (konzumacija hrane 2511,60 g, konačna masa 1585,36 g, randman trupa 68,13% i konverzije hrane 1.58).

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EFFECT OF USE OF RAW SOYBEAN IN THE FINAL MIXTURES FOR BROILER CHICKENS ON BODY WEIGHT, RELATIVE WEIGHT OF THE PANCREAS AND THE EDIBLE INNER ORGANS

V. Petričević[1], Ž. Jokić[2], M. Lukić[1], Z. Škrbić[1], Z. Pavlovski[1], D. Vitorović[2], M. Petričević[1]

[1]Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080, Belgrade-Zemun, Republic of Serbia
[2]Faculty of Agriculture, Nemanjina 6, 11080, Belgrade-Zemun, Republic of Serbia
Corresponding author: veselin5@live.com
Original scientific paper

Abstract: The aim of this study was to show the possibility of partial replacing of thermally treated soybean of standard variety "Lidija" and variety with reduced trypsin inhibitor "Lana" with raw grains. The effect of using different levels of participation of both raw soybean varieties, in the final mixtures for broiler chickens, on body weight of broiler chickens and the relative weight of the pancreas and edible internal organs (liver, heart and gizzard) was examined. The study was conducted according to the principle of two factorial experiment 2 x 5 (2 soybean varieties domestic breeding x 5 levels of participation of raw grain in the mixture) with a total of 10 dietary treatments. Obtained final body weights of broiler chickens in groups "Lana" I, II, III and IV were higher than the body weights of chickens which were fed the same level of raw grains of standard variety "Lidija". Differences in mortality rate between groups of broilers were not significant. The use of raw soybean grain resulted in hypertrophy of the pancreas. With the increasing participation of raw soybean in the diet gradually the relative weight of the pancreas increased in both varieties. The relative weight of liver and gizzard was not significantly influenced by the studied factors. In the "Lana" I and II the largest share of heart was established.

Key words: broiler chicken, soya, trypsin inhibitor, body weight, pancreas

Introduction

Soybean and its products are the most important protein components in poultry nutrition. Soybean is a valuable nutrient in terms of amino acid composition *(Tan-Wilson et al., 1987)*. Compared to all grain and legume species soy has the highest protein percentage (on average about 40%, with 2.1 to 2.5%

lysine), 14 - 27% of oil (with about 7% linolenic acid), 31-43% of carbohydrates (4-6% cellulose) and 4-6% ash. It is well known that the high nutritional value of soybean reduces the presence of a large number of anti-nutritional factors such as trypsin inhibitors, lectins, goitrogens, indigestible oligosaccharides, etc. Raw soybean grain in the diet of broiler chickens, in addition to the negative impact on the growth, also influences the increase of the relative weight of the pancreas. *(Arija et al., 2006)*. In order to avoid the negative effects of the use of raw soybean, before its use in the diet, present anti-nutritive substances must be inactivated. Different methods are used, but they are usually based on thermal treatment (wet and dry extrusion, micronization, etc.). In this way, the nutritional value of the feed is improved (higher digestibility of protein). However, it should be noted that the thermal treatments, in addition to use of large amounts of energy, may cause side effects, such as the Maillard reaction.

In order to reduce the cost of heat treatment of feed, through intensive breeding work, varieties were created with reduced content of certain anti-nutritional substances. As a result of the domestic soybean breeding program aimed at reduced trypsin inhibitor activity in our agricultural-environmental conditions, two varieties were created - "Lana" and "Luna" with lower levels of trypsin inhibitor. "Lana" is a late variety that yields equal to varieties of approximate duration of the vegetation, and ripe grain does not contain "Kunitz" trypsin inhibitor. In this way, there is a potential opportunity that one part of the protein in the diet is provided through the raw (untreated) kernels of mentioned domestic soybean varieties in final mixtures for broilers.

The aim of this study was to investigate the effects of replacing a portion of thermally treated soybean of standard variety "Lidija" and variety with reduced trypsin inhibitor "Lana" with raw soybean grain in the final mixtures for broiler chickens.

Material and methods

The study was conducted at the experimental farm of the Institute for Animal Husbandry in Zemun, using broiler chickens of heavy line hybrid Hubbard F15. In the nutrition of broiler chickens, in the final mixture was used two domestic varieties of soybean were used, variety "Lana" with reduced trypsin inhibitor and standard sort, "Lidija", thermally treated and untreated, that is raw (Table 1). Effect of different levels of raw soybeans in diets on final body weight of broiler chickens, the proportion of pancreas and edible internal organs were determined by factorial experiment 2 x 5 (2 soybean varieties x 5 levels of participation of raw grain in the mixture) with a total of 10 dietary treatments. Total of 2,000 day-old chicks were divided into 40 boxes, so there were 4 replicates per treatment.

Table 1. Level of trypsin inhibitor in soybean

Treatment	Raw soybean		Extruded soybean	
Variety	"Lana"	"Lidija"	"Lana"	"Lidija"
TI (mg/g/min)	17.71	36.74	4.38	14.03

Up to day 35 all animals in the trial were fed the exact same feed mixtures that were in line with the recommendations for the studied hybrid: starter (initial mixture), from 1 to 21 days and grower (mixture for growth), from 21 to 35 days. Finisher as a final mixture was available to broiler chickens from 36 to 42 days and differed for all the treatments in type and percentage of the used heat-treated and raw soybeans. From the 36th day on heat treated soybean in the control group was partially or completely replaced with raw kernels of the standard variety ("Lidija") and raw kernels of the variety with reduced trypsin inhibitor ("Lana") (25, 50, 75 and 100%). Feeding of chickens was organized so that food and water were available *ad libitum*. Ingredients and chemical composition of the mixture used in the experiment are given in Table 2

Table 2. Ingredients and chemical composition of the finisher mixtures for broilers used in the trial (%)

Feeds (%)	Finisher, from 36-42 day of age									
	"Lana"					"Lidija"				
	0% (K)	5% (I)	10% (II)	15% (III)	20% (IV)	0% (K)	5% (I)	10% (II)	15% (III)	20% (IV)
Extruded soybean-Lana	20	15	10	5	0	-	-	-	-	-
Raw Lana	0	5	10	15	20	-	-	-	-	-
Extruded soybean -Lidija	-	-	-	-	-	20	15	10	5	0
Raw Lidija	-	-	-	-	-	0	5	10	15	20
Corn	60	60	60	60	60	60	60	60	60	60
Sunflower meal	10	10	10	10	10	10	10	10	10	10
Yeast	4	4	4	4	4	4	4	4	4	4
Plant oil	2	2	2	2	2	2	2	2	2	2
Lime	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3	1.3
Mono calcium phosphate	1	1	1	1	1	1	1	1	1	1
Salt	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3	0.3
Mikozel	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4	0.4
Premix	1	1	1	1	1	1	1	1	1	1
Σ (%)	100	100	100	100	100	100	100	100	100	100
Chemical composition determined in laboratory analysis										
Protein	17.3	17.5	17.3	16.7	17.5	17.6	17.7	18.2	17.8	17.6
Moisture	10.9	11.6	11.7	12.1	12.1	11.4	11.8	11.8	11.9	11.9
Fat	8.31	7.94	8.09	8.03	8.98	7.75	8.25	8.15	8.83	8.16
Fibre	5.31	5.56	5.54	5.53	5.53	5.52	5.5	5.67	5.57	5.48
Ash	6.71	5.7	5.58	5.58	5.52	6.19	5.41	6.81	5.42	5.28
Ca	1.12	0.95	1.07	0.98	0.97	1.13	0.97	1.11	1.1	1.01
Total P	0.66	0.65	0.65	0.63	0.63	0.69	0.65	0.62	0.6	0.63
Na	0.22	0.2	0.21	0.22	0.19	0.21	0.2	0.21	0.2	0.19

The body weight of chickens was measured at the beginning (day 35), and at the end of the experiment (day 42). Mortality rate of chickens was determined on the basis of daily records of dead chickens. At the end of the trial, randomly, a total of 120 chickens were selected, 6 broilers per each treatment and sex. After a period of fasting of 12 hours, their body weights were measured and subsequently chickens were slaughtered. The relative weight of the pancreas, liver, heart and gizzard of all slaughtered chickens was determined.

The software package Statistica, version 6 (StatSoftInc.) was used for statistical analyses. The degree of statistical significance of differences between groups was determined by Tukey test.

Results and discussion

Average values of body weight of chickens at the age of 35 days (Table 3) indicate the uniformity of initial body weight in the trial between the formed groups. Chicken body weight, at the age of 35 days was in the range from1558 to 1607 g. Differences between initial body weights of chickens showed no statistical significance.

Table 3. Average body weight (g) and mortality (%)

Variety of soybean	Level of Raw soybean (%)	BW, day 35		BW, day 42		Mortality (%) u 6.nedelji
		x (g)	Sd	x (g)	Sd	
"Lana"	0%(K)	1572.54	204.18	2130.51^{ab}	254.00	0.00
	5%(I)	1574.67	214.00	2138.89^{ab}	282.64	0.50
	10%(II)	1574.47	195.49	2130.10^{ab}	280.33	1.53
	15%(III)	1568.32	223.59	2068.57^{bc}	300.02	0.50
	20%(IV)	1586.01	207.73	2081.89^{b}	277.92	1.00
"Lidija"	0%(K)	1607.09	237.99	2202.32^{a}	341.57	1.02
	5%(I)	1558.87	205.40	2126.72^{ab}	273.21	1.53
	10%(II)	1593.32	201.89	2104.23^{b}	276.24	1.01
	15%(III)	1589.64	216.39	2056.34^{bc}	292.48	0.52
	20%(IV)	1594.44	191.16	2041.03^{c}	236.45	1.51

* a,b,c Average values in each column without superscripts are significantly different at the level of 5%

Broiler chickens in the "Lydia"-K group, fed mixtures with 0% raw soybean of variety "Lidija", had the highest body weights (2202.32 g), which were significantly higher ($p < 0.05$) than in group II, III and IV of the same variety (2104.23g, 2056.34g and 2041.03g) and group III and IV of varieties "Lana" (2068.57g and 2081.89g).

The trend of a gradual decrease in final body weight of broiler chickens with the increase of the level of participation of raw soybeans in mixtures is particularly expressed in chickens fed diets containing soybean variety "Lidija". This is confirmed by the results of *Beuković et al. (2008).*

Introduction of raw soybeans with low levels of trypsin inhibitor ("Lana") in the diet of broiler chickens in the final stage, exhibited positive effect on body weight compared to conventional soybean variety ("Lidija"), but much weaker effect than the heat-treated soybeans. This is confirmed by the results of following authors (*Han et al., 1991, Anderson-Hafermann et al., 1992,. Douglas et al., 1999, Palacios et al., 2004, Jokić et al., 2004*) in trials with chickens and *Zhang et al. (1991)* with the layer hens.

In the studies by *Beuković et al. (2009),* the share of thermally unprocessed soybeans without the Kunitz trypsin inhibitor can be used in diets for older categories of pigs, but at concentrations no greater than 15%, otherwise it leads to a reduction in daily gain.

On the basis of data on mortality of chickens in the final week of the trial (Table 3) it can be concluded that the chickens of all groups in the experiment showed high vitality, as evidenced by very low mortality values. Analysis of the impact of soybean varieties and levels of participation of raw soybeans in mixtures on mortality rate showed no significant effect of the examined factors.

Table 4. Relative mass and length of digestive organs

Variety of soybean	Level of Raw soybean (%)	Pancreas		Liver		Heart		Gizzard	
		x (%)	Sd	x (%)	Sd	x (%)	Sd	x (%)	Sd
"Lana"	0%(K)	0.21^{y}	0.02	2.15	0.20	0.66^{a}	0.07	1.81	0.21
	5% (I)	0.21^{y}	0.03	2.12	0.24	0.65^{a}	0.06	1.92	0.26
	10%(II)	0.23^{y}	0.02	2.14	0.25	0.62^{ab}	0.06	2.03	0.22
	15%(III)	0.26^{y}	0.03	1.87	0.17	0.58^{ab}	0.07	1.91	0.22
	20%(IV)	0.27^{y}	0.07	2.05	0.31	0.63^{ab}	0.08	2.06	0.31
"Lidija"	0%(K)	0.22^{y}	0.02	1.98	0.34	0.56^{b}	0.04	1.80	0.17
	5%(I)	0.26^{y}	0.04	1.96	0.18	0.60^{ab}	0.08	1.95	0.27
	10%(II)	0.28^{y}	0.04	2.01	0.26	0.56^{b}	0.05	2.07	0.31
	15%(III)	0.28^{y}	0.08	2.00	0.16	0.60^{ab}	0.06	1.97	0.27
	20%(IV)	0.36^{x}	0.11	2.10	0.25	0.62^{ab}	0.07	1.98	0.30

* x-y Average values in each column without superscripts are significantly different at the level of 1%
* a-b Average values in each column without superscripts are significantly different at the level of 5%

The relative weight of the pancreas and edible internal organs are shown in Table 4. Raw soybeans in the final mixtures for chickens resulted in hypertrophy of the pancreas. With the increasing participation of raw soybean in the diet, gradually the relative weight of the pancreas increased in case of both soybean varieties. Statistically significantly higher $p<0.01$ relative weight of the pancreas was determined in chickens of the "Lidija" IV group compared to all other groups. These results are consistent with the results of *Beuković et al., (2010).* The relative liver weight was not significantly influenced by the factors examined which is contrary to the results of *Beuković et al., (2010).* Share of heart in groups "Lana" I and II was significantly higher $p<0.05$ compared to the group "Lidija" K and "Lidija" I.

Conclusion

The inclusion of raw soybean of variety "Lana" with the participation of up to 10% and raw soybean variety "Lidija" with the participation of up to 5% in the final mixture did not have negative effect on the final body weights. The average final body weight of fattening chickens in groups III and IV of both varieties was significantly lower than in the control group of variety "Lidija". The increase of the share of raw soybeans reduced the final body weight of fattening chickens. The inclusion of raw soybeans did not have negative effect on the mortality rate of chickens.

With the increasing participation of raw soybeans the share of the pancreas increased. Hypertrophy of the pancreas is particularly pronounced in the group with the maximum participation of raw soybeans of standard variety. The relative liver weight was not significantly influenced by the studied factors.

Extrusion process (heat treatment) neutralizes the anti-nutritive substances from soybeans and hence the negative effects. Variety "Lana" did not produce satisfactory results in terms that raw kernels of this variety can completely replace heat treated soybean, but the results were better in comparison to raw soybeans with a standard level of trypsin inhibitor.

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Efekat upotrebe sirove soje u završnim smešama za ishranu brojlerskih pilića na telesne mase, relativnu masu pankreasa i jestivih unutrašnjih organa

V. Petričević, M. Lukić, Ž. Jokić, Z. Škrbić, Z. Pavlovski, D. Vitorović, M. Petričević

Rezime

Cilj ovog istraživanja bio je da ukaže na mogućnost zamene jednog dela termički obrađenog sojinog zrna standardne sorte „Lidija“ i sorte sa smanjenim sadržajem tripsin inhibitora „Lana“, sirovim zrnom. Ispitan je efekat korišćenja različitog nivoa učešća sirove soje obe sorte na telesnu masu brojlerskih pilića i relativne mase pankreasa i jestivih unutrašnjih organa (jetra, srce i želudac) u završnim smešama za ishranu tovnih pilića.

Istraživanje je izvedeno po principu dvofaktorijalnog ogleda 2 x 5 (2 sorte soje domaće selekcije x 5 nivoa učešća sirovog zrna u smeši) sa ukupno 10 tretmana ishrane.

Dobijene završne telesne mase brojlerskih pilića u grupama „Lana“ I, II, III i IV su bile veće u odnosu na mase pilića koji su koristili isti nivo sirovog zrna standardne sorte „Lidija“. Razlike u mortalitetu među grupama brojlera nisu bile značajne. Upotreba sirovog zrna soje uticala je na hipertrofiju pankreasa. Sa povećanjem učešća sirove soje u obroku postepeno se povećavala relativna masa pankreasa kod obe sorte. Relativna masa jetre i želudca nije bila pod značajnim uticajem ispitivanih faktora. U grupama „Lana“ I i II utvrđen je najveći udeo srca.

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POSTER SECTION III

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THE RUSSIAN HISTORY OF DAIRY GOAT FARMING

T.A. Magomadov, V.A. Shatalov

Russian State Agrarian University named after K.A. Timiryazev, Moscow, Russia
Corresponding author: dairygoatfarming@gmail.com

Abstract: In article the most significant periods in development of a dairy goat's farming in our country are considered. The Society of dairy goat breeding attempted to engage people of great possessions in breeding of high-profitable foreign breeds on the farm. They were hoping this livestock would affect the Russian goat breeding in general. The number of goats in USSR became to decline. However no valuable attempts to develop the goat breeding were made. At postwar period The Soviet Government tried to raise the dairy goat farming to the high level. The main emphasis was on fiber and wool goat farming. There were many goats' fiber and wool recycling plants and factories. They didn't pay attention to meat goat farming because they considered that lamb meat more nutritious and tasty. The dairy goat farming wasn't discussed because goat's milk wasn't popular that time. Nowadays in Russian Federation domestic goats are breed on private farms of citizens rather than in agricultural enterprises. The need in enhanced studying of goat farming is very actual and very high especially for leading regions in it. Development of dairy goat farming in commercial way is scotched by absence of normative documentation of goat's milk. Nowadays Russia hasn't got any Federal or Local standards on this production. It makes sales through the retail impossible.

Keywords: Saanen and Toggenburg breeds, society, heifer project international.

Introduction

Goat breeding is an important branch of animal husbandry. Number of goats in the world in recent years, has been increasing. In addition to the countries of Asia and Africa, where it is concentrated the largest number of goats, goats have been successfully grown in Europe and America. Goat milk is highly prized product. Goat is also interesting for Russia because there are very favorable conditions for the development of this branch of farming *(Erohin et al., 2001,*

Kolosov et al., 2001,Perevozcikov, 2006, *Shatalov, 2013).*This paper presented historical component of goat farming in Russia.

History of dairy goat

The main characteristic of dairy goat's farming history is a permanent rotation from progress to regress in rather periods of development. The latest rise of it was at the end of nineteenth century in France and then in Germany. After that it became popular in the North America.

At the end of the nineteenth century the dairy goat farming in Russia was promoted much but still it wasn't popular. At that time poor farmers were managing nondescript and underproductive goats. That's why there was a proverb: The goat is a cow of a poor man.

The Society of dairy goat breeding attempted to engage people of great possessions in breeding of high-profitable foreign breeds on the farm. They were hoping this livestock would affect the Russian goat breeding in general. The Society organized exhibitions with a touch of imagination in Saint Petersburg's Gardens and gave out tastes of goat's milk and some products from it. Also they organized excursions to farms of Grand Ducal's estates. But they still got nowhere. Only breeds horses, cows and birds farming were popular then. One of Society's enthusiasts wrote: «We are sleeping like dogs here, whereas abroad the goats are cultivated to the utmost. Slow down, unbelievers. It's nobody's dream to substitute airplanes and engines, marching on the streets with goats. What is meant here is the nation's nutrition».

In 1911 Duke Urusov published the book «The goat. Breeding, care and farming». It turned out to become a very successful manual on farming of dairy goats which rendered an invaluable service for economies of many countries. They gave milk, cheese, wool, leather and fur. The great achievement of author was promotion of dairy goat farming as a main way of farming welfare.

The result of his third publication was decision of Ministry of the Interior to import big population of pedigree Saanen and Toggenburg goats from Switzerland to the Central and North-European parts of Russia. It ended up with the local breeds to be improved and a huge long-term work on selection of the new goat breeds to be started.

At that time slaughterhouse's veterinarians found out that goats hardly ever suffered from tuberculosis. Specialist inspection proved that goats had good resistance to other terminal sicknesses. By that time physicians were trying to find breast milk substitute. In scientist's opinion it could became goat's milk.

Special attention was devoted to examining goat's milk. Austrian and German sociologists had find out that there was correlation between the level of

dairy goat's farming development and child mortality in different regions. Statistics showed: the more goats to be farmed, the less percentage of child mortality could be.

Russian physicians (generally in Saint Petersburg) assigned goat's milk to patients who suffered from catarrhal and pneumonic diseases. The demand on it increased dramatically. The farms didn't have enough fresh goats' milk and sold it only by doctor's recipes.

During the next hard times for the country the dairy goat farming was distressed and didn't have enough attention till 1935.

Development of textile industry was the time of big purchases of expensive goat's wool abroad. This made the Russians to think of its own raw materials base. In 1936 700 heads of Angora goats were imported from USA. It helped breeding of new wool breeds in Kazakhstan, Tajikistan, Uzbekistan and other Soviet Union's Republics.

The Second World War influenced on this area badly. The number of goats in USSR became to decline. However no valuable attempts to develop the goat breeding were made.

At postwar period The Soviet Government tried to raise the dairy goat farming to the high level. The main emphasis was on fiber and wool goat farming. There were many goats' fiber and wool recycling plants and factories. They didn't pay attention to meat goat farming because they considered that lamb meat more nutritious and tasty. The dairy goat farming wasn't discussed because goat's milk wasn't popular that time.

In the nineties of the twentieth century such a large breed as Saanen was under threat of degeneracy. High-quality breeders which were imported in Soviet times from Germany and New Zealand after breeding huge get exhausted their resources. The farmers had to start inbreeding process which is known to cause negative effect to the livestock. The USA specialists gave advice to their colleagues in Leningrad region to address to Heifer International Project which was founded in 1944. It was a non-commercial organization which helped people in more than one hundred countries of the world. Heifer International Project distributed animals without regard to race, religion and political convictions. The main condition was to share the private posterity with people in need.

In November of 1996 the request about importing of two Saanen goats in Leningrad region was sent to the main office to USA. In 1999 after long lasting negotiations and the visit of Heifer International Project's delegate Sew Bertrand the project was opened.

In period between 1999 till 2002 Heifer International Project made donation of ninety five goats. Also they taught Russian farmers to care and hold goats, held training in veterinary medicine and milk processing. Project participants

were provided with implements and veterinary preparations. Project's goats often won exhibitions. The dairy goat farming magazine was founded for wide expansion.

If we compare statistics of producing goat's milk in Russia since 1992 till 2010 we will see that climax was reached in 2000. It's three hundred sixteen million and five hundred and forty one thousand tons of milk. It's more than in 2010 in sixty one millions and three hundred sixty six thousand tones of milk (see Figure 1).

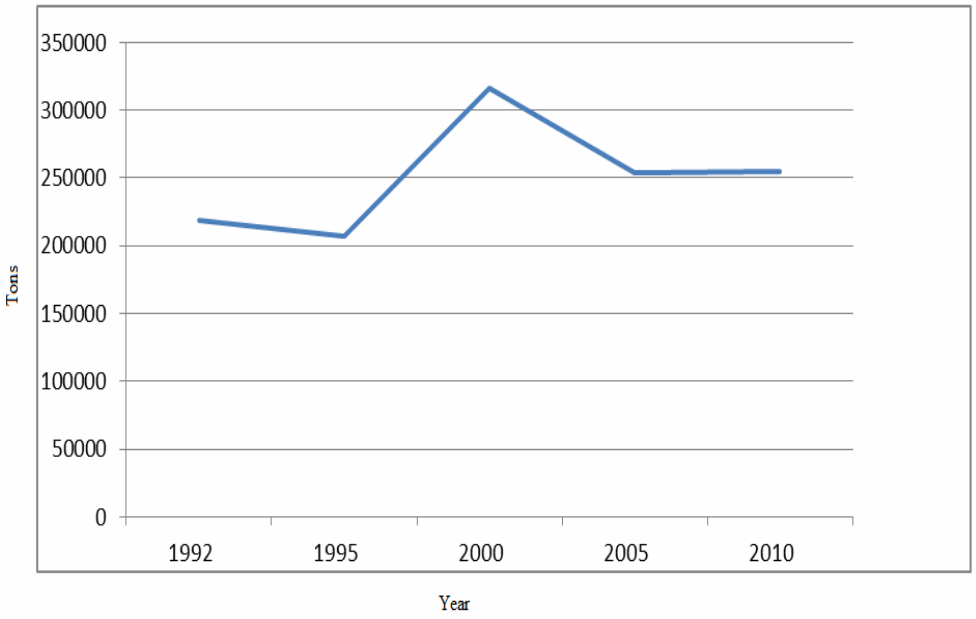

Figure 1. The dynamics of producing goat's milk in Russia

The dynamics of producing goat's milk in the world is rather different. At period since 1990 till 2008 we can see growth of producing since nine thousands and ninety hundred eighty tons till fifteen thousands and two hundred fifteen tones (see figure 2).

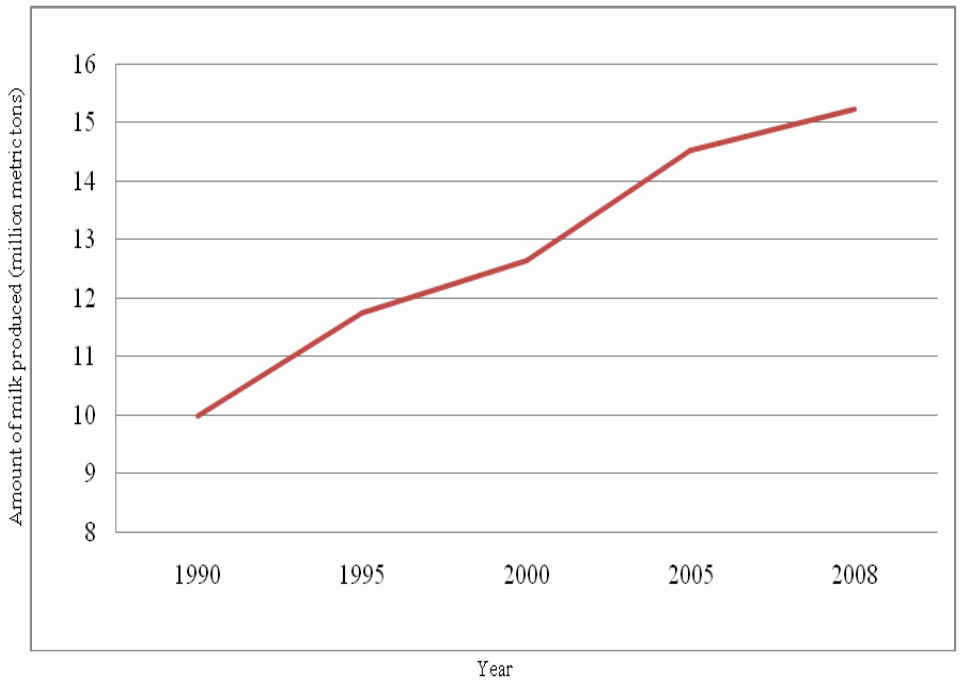

Figure 2. The dynamics of producing goat's milk in the world (*FAOSTAT, 2008*)

Nowadays in Russian Federation domestic goats are bred on private farms of citizens rather than in agricultural enterprises *(Erohin et al., 2001, Perevozcikov, 2006, Smirnov, 2002, Sokolov, 2004*). The need in enhanced studying of goat farming is very actual and very high especially for leading regions in it *(Shatalov, 2013).*

Development of dairy goat farming in commercial way is scotched by absence of normative documentation of goat's milk. Nowadays Russia hasn't got any Federal or Local standards on this production. It makes sales through the retail impossible.

Conclusion

The main characteristic of dairy goat's farming history is a permanent rotation from progress to regress in rather periods of development

The Society of dairy goat breeding attempted to engage people of great possessions in breeding of high-profitable foreign breeds on the farm. They were hoping this livestock would affect the Russian goat breeding in general.

If we compare statistics of producing goat's milk in Russia since 1992 till 2010 we will see that climax was reached in 2000. It's three hundred sixteen million and five hundred and forty one thousand tons of milk. It's more than in 2010 in sixty one millions and three hundred sixty six thousand tons of milk.

Development of dairy goat farming in commercial way is scotched by absence of normative documentation of goat's milk. Nowadays Russia hasn't got any Federal or Local standards on this production. It makes sales through the retail impossible.

Istorijat gajenja mlečnih rasa koza u Rusiji

T.A. Magomadov, V.A. Shatalov

Rezime

U radu su prikazani najznačajniji periodi u razvoju mlečnog kozarstva u našoj zemlji. Društvo za uzgoj mlečnih koza je pokušalo da angažuje ljude dobrog imovinskog stanja u uzgoju visoko-profitabilnih stranih rasa na farmama. Nadali su se da će ova stoka uticati na ruski uzgoj koza u celini. Broj koza u SSSR počeo je da opada. Međutim, nije bilo značajnih pokušaja da se razvije uzgoj koza. U posleratnom periodu, sovjetska vlada je pokušala da podigne uzgoj mlečnih koza na visoki nivo. Glavni akcenat je bio na proizvodnji vlakana i vune u kozarstvu. Bilo je mnogo postrojenja za reciklažu vune i vlakana koza, kao i fabrika. Nije se obraćala pažnja na proizvodnju kozjeg mesa, jer su smatrali da je jagnjeće meso hranljivije i ukusnije. Mlečno kozarstvo se nije ni razmatralo, jer kozje mleko nije bilo popularno u to vreme. Danas u Ruskoj Federaciji domaće rase koza se gaje na privatnim gazdinstvima građana, a ne u poljoprivrednim preduzećima. Potreba za poboljšanim ispitivanjem kozarstva je veoma aktuelna i veoma visoka, posebno za vodeće regione. Razvoj poljoprivrede mlečnih koza na komercijalni način je usporen zbog odsustva normativne dokumentacije koja se odnosi na kozje mleko. Danas Rusija nema nikakve savezne ili lokalne standarde o ovoj proizvodnji, što maloprodaju čini praktično nemogućom.

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THE EFFECT OF DIFFERENT PROTEIN SOURCES IN THE DIET ON GOAT PRODUCTION PERFORMANCE DURING FATTENING

J. Stojković[1], Z. Ilić[1], M.P. Petrović[2], V. Caro Petrović[2], I. Đorđević[3], S. Ćirić[1]

[1]University of Pristina, Faculty of Agriculture in Kosovska Mitrovica, Republic Serbia
[2]Institute for Animal Husbandry, 11080 Belgrade, Republic of Serbia
[3] spec.ag. eng., Association for the Animal Welfare Pastir, Nis
Corresponding author: Jovan Stojković, e-mail: jovanips@gmail.com
Original scientific paper

Abstract: This paper presents the results of the effect of different protein sources in the diet on growth intensity and the usage of foods and nutrients for weaned goats during intensive fattening. The experiment was carried out on 60 Balkan kid goats, weaned at 60 days, and divided into 3 groups. The goats were fed with concentrate mixture and alfalfa hay was available at will. We examined the effect of using three mixtures which differed in terms of the percentage of non-degradable protein at the rumen level: 56% (I), 52% (II) and 49% (III), which was achieved by using different protein sources: soy meal (I), sunflower meal (II) and rapeseed meal (III), on goats' production parameters during fattening. Statistical analysis was performed by using Statistica program, version 6, Stat.Soft, Inc. (2003). Body mass at the beginning of the experiment was: 19,50 kg (I), 19,45 kg (II) and 19,50 (III). Body mass at the end the experiment was: 36,10 kg (I), 34,50 kg (II) amd 31,70 kg (III). Goats on I: II: III diet type realized average daily weight gain: of 0,276:0,250:0,203 kg, respectfully. Conversion of feed mixture (kg / kg of weight gain) on analogue treatment was: 3,10:3,35:3,70, of energy (NEM MJ / kg) was: 27,90:30,69:33,17, and of total protein (g/kg) was: 570:597:679.

Key words: kid goats, weight gain, protein source, feed conversion

Introduction

Proteins are one of the most critical nutrients in the diet of domestic animals, especially young animals with fast growth. Therefore, the protein feed must be used rationally, because it is usually much more expensive than energy feeds, leading to an increase in feeding costs. Particular importance in the nutritive concept of fattening, apart from the adequate supply of energy, is given to protein

meals. In recent years, goats feeders devote more attention to protein sources in the diet, especially in terms of the degree of degradation in the rumen, that is of the amount of amino acids that are provided at the level of the duodenum. Active microbial population in reticulum-rumen breaks down the proteins in the meal into to peptides, amino acids and ammonia, and then uses these materials to synthesize their own proteins. In each of these breakdown and synthesis processes certain losses occur which finally means that a changed amount of amino acids reaches the point of digestion.

A large amount of protein for kid goats, whose specific amino acid requirements are met by microbial fermentation of substances in the rumen, comes from plant sources. The protein content in plants varies considerably from one plant species to another. Even within the same plant species there are significant differences between the phases of vegetation or different parts of the plant. Proteins in plants are related to the plant tissue, such as leaves, growth centers and fruits, in which an active metabolism of proteins takes place, and they must have the potential to metabolize nutrients at certain critical stages of development.

Material and Methods

Studies were carried out on a goat farm in Matejevac, a village near the town of Niš. For the research we used Balkan kid goats. Feeding study encompassed a total of 60 kid goats, weaned at 60 days and divided into 3 homogeneous groups. The average body weight of kid goats at the beginning of the study was around 19.48 kg, and at the end of the study was 34.10 kg. Feeding kid goats with hay and forage mixtures was done at the group level and the food was available at will. We examined three feed mixtures and the basic differences between them were conditioned by the desire to provide different proportion of non-degradable protein sources when choosing the protein value of concentrated foods: soybean meal (diet type I), sunflower meal (diet type II) and rapeseed meal (diet type III). The body weight of kid goats in the study was determined by measuring them at an interval of 15 days. Feed conversion was calculated according to the growth and food consumption between the two measurements. Nutrient values were determined based on the French system recommended by (*Stojković, 2004; Memiši et al., 2008)*. The structure of used mixtures is shown in Table 1, and the nutritive value in Table 2.

Table 1. Structure of concentrate mixtures for fattening of weaned kids, %

Feeds	Concentrcate mixtures		
	I	II	III
Maize	75	73	76
Soy bean meal	21	-	-
Sunflower meal	-	23	-
Rapeseed meal	-	-	20
Limestone	2	2	2
Salt	1	1	1
Premix	1	1	1
TOTAL	100,00	100	100

Statistical analysis was done using the *Stat.Soft, Inc. (2003*) computer program Statistica (data analysis software system) version 6, which uses standard mathematical statistical methods.

Results and Discussion

The data on average production indicators for dietary treatments are shown in Table 3.

Body weight and weight gain: a protein source in concentrate mixtures used, that is the level of non-degradable protein in the total protein, had a highly significant (P0< 01) influence on the final fattening effect expressed by an average daily gain of 0.276 kg (I), 0.250 kg (II) and 0.203 kg (III) and the final body weight of the studied animals: 36.10 kg (I), 34.50 kg (II) and 31.70 kg (III). It is known that in ruminants the active bacteria population breaks down protein meal in the rumen into peptides, amino acids and ammonia, and then uses these materials for the synthesis of its own proteins. In each of these breakdown and synthesis processes certain losses occur (usually about 20% but sometimes higher), which means that through the action of microorganisms a lower amount of amino acids reaches the point of digestion.

If the extent of breaking-down is larger, the amount of amino acids which is directly acquired by the animal is proportionally smaller *Memiši et al., (2007)*. The specific role of the absorbed amino acids from the bowel of kid goats is the protein synthesis which contributes to the increase of bodily tissues. This is particularly important in the intensive fattening of the weaned kid goats that are characterized by high genetic capacity for growth. Therefore, feeds which proteins have passed through the reticulum-rumen largely un-degraded and got into duodenum (soybean meal and sunflower meal) allowed for a higher weight gain in

kid goats. The confirmation of these results can be found in the research of (*Žujović et al., 2006*; *Memiši and, Bauman 2007*; *Stojković 2004*).

Table 2. Nutritive value of used concentrate mixtures in fattening of weaned kids

Nutrition parameters	Concentrate mixtures		
	I	II	III
Dry matter, %	86.9	87.2	86.9
OFU	1.2	1.2	1.2
NEM, MJ/kg	7.49	7.68	7.79
UFV	0.89	1.07	1.05
Total protein, g/kg	140	136	134
NP, %	73	69	63
NP in CP.	56	52	49
PDIN, g/head/day	105	103	101
PDIE, g/head/day	116	113	106
Ashes	26	25	24
Ca, g	9.7	8.5	8.2
P, g	4.9	4.1	4.0

Table 3. Production results of kids in fattening

Parameters	TYPE OF NUTRITION		
	I	II	III
Body mass at the beginning of trial, kg	19.50	19.45	19.50
Age at the beginning of trial, days	60	60	60
Body mass at the end the trial, kg	36.10	34.50*	31.70**
Total gain, kg	16.60	15.05*	12.20**
Average daily gain, kg	0.276	0.250*	0.203**

** (P<0,01); *(P<0,05)

Table 4. Consumption of feed an nutritive substances per kg of kids gain (kg/kg)

Feeds	TYPE OF NUTRITION		
	I	II	III
Concentrate mixture, kg/kg	3.10	3.35	3.70
Alfalfa hay	1.15	1.30	1.45
DM	3.80	3.90	4.32
CP, g/kg	570	597	679
PDIM, g/kg	598	636	689
PDIE, g/kg	410	470	495
NEM, MJ/kg	27,90	30.69	33.17

Food and nutrients consumption: Results of food and nutrients consumption for one kilogram of weight gain for kid goats are shown in Table 4. The best dry matter conversion was observed with the kid goats that were treated

to soybean meal (I) whose consumption of feeds per kg of weight gain was 12.10% lower compared to kid goats treated to rapeseed meal (III) and 3.18% lower as compared to sunflower meal treatment (II). Also, minimum dry matter consumption per unit of weight gain was observed in kid goats with diet type I, who, compared to animals of type II and III, used 6.10% and 13.34% less protein. With the increasing use of non-degradable protein in the total protein, there was a reduction in energy consumption of 4.5 to 7.1 MJ NEM for each kilogram of weight gain. Data of feed and nutrients conversion obtained in these studies are consistent with *Ugur et al. (2004), Žujović et al. (2001*) and *Stojković (2004*), who found that, depending on the protein source in the concentrate mixtures or on the higher percentage of the non-degradable protein in the total protein meals, the consumption of foods and nutrients for the unit of the achieved growth becomes lower.

Conclusion

Based on the results obtained by studying the effect of protein source in the diet on production results during the fattening of kid goats, the following conclusions may be drawn:

1. The highest average daily gain (0.276 kg) was recorded for the goats treated to soybean meal (the non-degradable protein percentage at the level of rumen was 56% of the total).

2. With the reduction of non-degradable protein level in meals (56:52:49%), the consumption of dry matter, total protein and energy per kilogram of gain become increased. The optimum conversion was observed for kid goats treated to soybean meal as a source of protein.

3. The obtained results are illustrative in terms of the optimal percentage of non-degradable proteins at the level of goat rumen and suggest that the best performance was achieved by the kid goats with the diet type I with 56% of NP (non-degradable proteins) in the concentrate mixture.

Acknowledgment

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Efekat izvora proteina u obroku na proizvodne rezultate jaradi u tovu

J. Stojković, Z. Ilić, M.P. Petrović, Violeta Caro Petrović, Igor Đorđević, Slavica Ćirić

Rezime

U radu su prikazani rezultati uticaja različitih izvora proteina u obroku na intenzitet porasta i iskorišćavanje hrane i hranljivih materija kod odlučene jaradi u intenzivnom tovu. Ogled je izveden na 60 jaradi Balkanske koze, zalučenih sa 60 dana uzrasta, podeljenih u 3 grupe. Ishrana grla koncentrovanim smešama i lucerkinim senom bila je po volji. Ispitivan je efekat korišćenja tri smeše koje su se razlikovale u pogledu udela nerazgradivog proteina na nivou buraga: 56% (I), 52% (II) i 49% (III), što se postiglo korišćenjem različitih izvora proteina: suncokretova sačme (I), sojine sačme (II) i sačme uljane repice (III), na proizvodne parametre jaradi u tovu. Statistička obrada dobijenih podataka je izvršena na računaru korišćenjem programa Stat.Soft, Inc. (2003). Jarad na tipovima ishrane I:II:III su ostvarila prosečan dnevni prirast: 0,276:0,250:0,203 kg. Konverzija krmne smeše (kg/kg prirasta) na analognim tretmanima je iznosila: 3,10:3,55:3,70, energije (MJ NEM/kg): 27,90:30,69:33,17, ukupnih proteina (g/kg): 570:597:679.

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STUDY ON THE EFFECT OF SOYPASS ON THE SHEEP MILK PRODUCTION

T. Ivanova, E. Raicheva

Institute of Animal Science – Kostinbrod, 2232, Bulgaria
Corresponding author: t_st_ivanova@abv.bg
Original scientific paper

Abstract: The aim of the study was to test and evaluate the effect of the replacement of the traditional sunflower meal with SoyPass on the milk production of sheep. The experiment was conducted with 40 ewes of the Synthetic population Bulgarian milk from the flock of IAS – Kostinbrod, Bulgaria. The duration of the experiment was 180 days. The adaptation period was 15 days prior to giving birth. The sheep were divided in two groups (control and experimental). The animals from the both groups were equalized according to age, number of lactation, milk production and type of birth. The sheep were fed according to the standards and the feed consumption was controlled daily. In addition to the concentrate, the experimental group received 7,5% SoyPass and 7,5% sunflower meal, and the control – 15 % sunflower meal. The milk production was tested every 15 days. The test day milk was determined according to the AC method of ICAR. The composition of the milk – fat and protein content (%), and dry matter (%), were determined for a 20-ml sample of milk using a milk analyzer Ecomilk, Bultech Company. The information obtained was evaluated using the methods of variation statistics. The significantly higher milk production for a standard 120 days milking period in the experimental animals that was observed can be explained by the presence of SoyPass in the ration. The values for the studied milk contents - milk fat and dry matter, were significantly higher in the control group ($P<0.05$; $P<0.01$). The values for the protein content were significantly higher in the experimental group ($P<0.05$; $P<0.01$).

Key words: dairy sheep, feeding, Soypass, sunflower meal, milk production

Introduction

The milk sheep in Bulgaria form the highest percentage of the sheep breed in our country and the studies on the improvement of their productivity by using various feeding strategies is of specific scientific interest. Studies of the effect of

different feed additives to the ration have been carried out in Bulgaria *(Raicheva and Ivanova, 2009; Surdzhiyska et al., 2004; Shindarska et al., 2003; Kozelov et al., 2001).* There is still no information in our specialized publications concerning the effects of different feeding strategies on the milk productivity of sheep, which could provoke the conducting of such type of experiments. This was the first study on the effect of the protected protein SoyPass in dairy sheep. SoyPass is produced through unique patented process which doubles the amount of bypass protein in soyabean meal. SoyPass increases the bypass capacity of protein, by binding the soya protein with sugars, causing the Maillard reaction. This reaction binds the sugars to the protein thus creating a bond which the rumen microorganisms cannot break. This bond is a physical blockage to the microbial enzymes, preventing them from binding to the protein chain, and hence from acting in their accepted way. The mechanism by which the protected proteins are digested in the abomasum and small intestine is of course the same mechanism which is used for unprotected proteins.

The aim of the study was to test and assess the effect of the replacement of traditional sunflower meal with SoyPass on the milk production of sheep.

Materials and Methods

The experiment was conducted with 40 ewes of the Synthetic population Bulgarian milk (SPBM) from the flock of IAS – Kostinbrod, Bulgaria. The duration of the experiment was 180 days. The sheep were divided in two groups (control and experimental) each containing 20 ewes. The animals were of the same age, number of lactation, milk production and type of birth.

Feeding of the sheep was in groups, according to the standards *(Todorov and Dardjonov, 1997)*, with constant access to water. The feed consumption was daily controlled. The quantity and composition of the ration of the experimental and control groups are shown in tables 1 and 2. In addition to the concentrate, the experimental group received 7,5% SoyPass and 7,5% sunflower meal, and the control – 15 % sunflower meal. The adaptation period was 15 days prior to giving birth.

The milk production was tested every 15 days at the department of Sheep breeding. The test day milk was determined according to the Instruction on the Control of the Productive Qualities (2003) by the AC method of ICAR. The individual quantity of milk of the each ewe was controlled. The quantity of the milk was measured in volume measurement units (ml). The milk production of each ewe for the test day was calculated by multiplying the quantity of the milked milk at the individual test in the morning by the coefficient for the herd, determined for the test day with reference to the quantity of the morning and

evening milk : the morning milk. The milk of each sheep for one test period was calculated by multiplying the milk production for the test day by the number of days in the test period. The milk production for the 120 days milking period for each sheep was determined by the sum of the milk production for the individual test periods.

The composition of the milk – fat and protein content (%), and dry matter (%), were determined for a 20-ml sample of milk using a milk analyzer Ecomilk, Bultech Company.

The information obtained was evaluated using the methods of variation statistics.

Table 1. Ingredients of the daily diet

Ingredients, kg	
Alfalfa hay	1.3
Corn silage	2.0
Concentrate mixture	1.0

Table 2. Ingredients of the concentrate mixture

Ingredients, %	Control	Experimental (SOYPASS)
Corn	12.0	12.0
Wheat	12.0	12.0
Triticale	36.8	36.8
Fermodil	21.5	21.5
Sunflower meal	15	7.5
SOYPASS	-	7.5
Salt	0.7	0.7
Dicalcium phosphate	0.7	0.7
Calcium carbonate	1.2	1.2
Premix	0.1	0.1

Results and Discussion

During the experiment the ewes of the control and the experimental groups were fed at the ration of 4.3 kg (table 1). The voluminous fodders – alfalfa hay and corn silage were respectively, 1.3 kg and 2.0 kg, while the concentrate – 1.0 kg per ewe.

The composition of the concentrate (table 2) for the two groups differed between the groups - the experimental group received 7.5% SoyPass and 7.5% sunflower meal, and the control – 15 % sunflower meal. The rest of the components – corn, wheat, triticale and Fermodil were in equal percentage ratios. The chemical composition and feeding value of feeds were shown in table 3.

Table 3. Chemical composition and feeding value of feeds

Items	**Forages**		
	Meadow hay	**Corn silage**	**Concentrate mixture**
Dry matter, %	114.2	58.2	84.41
% of the DM			
Crude protein	9.7	2.6	17.18
Crude fibers	26.3	6.9	8.40
Ether extract	1.5	0.8	3.21
NFE	48.3	15.8	52.3
Ash	5.8	2.3	3.4
1 kg DM contain:			
FU	0.64	0.94	0.95
FUM	0.79	0.99	1.02
CP, g	115	84	145
PDI, g	75.,6	66	104
BPR, g	- 3.4	- 18.5	- 2.53
BPR/ 1 FUM	- 4	- 17	- 1.8

The daily dry matter intake of the sheep from the control group was 2,6 kg, containing 2,4 FUM of energy and that of experimental group, respectively, 2,61 kg and 2,37 FUM (table 4). The daily protein intake (CP, PDI and BPR, g) was respectively, 440 g, 203 g and 70 g for the control, and 432 g, 215 g and 54 g for the experimental group.

Table 4. Dry matter, energy and protein intake

Items	Control	Experimental (SOYPASS)
Dry matter intake- total, kg	2.6	2.61
Energy intake – total, FUM	2.4	2.37
Protein intake – CP, g PDI, g BPR, g	 440 203 70	 432 215 54

Note: FUM - Feed Unit for milk, CP - Crude protein, PDI - Protein digestible intestine, BPR - Balance protein ruminant

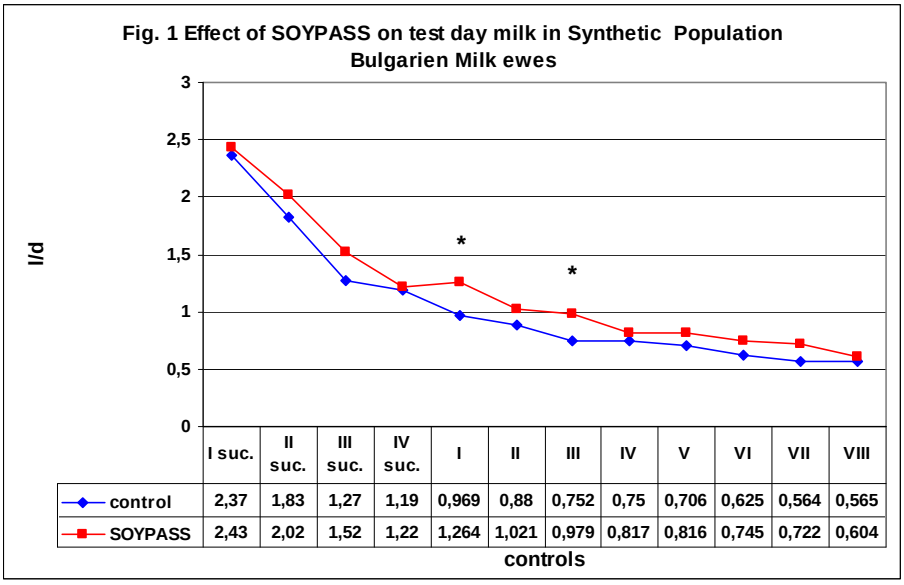
Table 5. Values of the F-criterion for the effect of Soypass on the milk production in the ewes, n=40

Traits	F- criterion
Milk production from suckling controls	
I	NS
II	NS
III	NS
IV	NS
Milk production from milking controls	
I	6.46 *
II	NS
III	5.26 *
IV	NS
V	NS
VI	NS
VII	NS
VIII	NS
120 days milking milk production	5.51 *

Note: Significant at: * - $P<0.05$

The values of the F-criterion for the effect of SoyPass on the milk production in the ewes from Synthetic population Bulgarian milk are presented in table 5. There was significant effect for first and third milking controls (P<0.05). The significant value of the F-criterion for the effect of SoyPass was determined at the 120 days milking milk production too (P<0.05).

The shape of the curves is similar for both groups (fig. 1). With exception of the first suckling, last suckling and the last milking controls in the experimental ewes, there was a tendency for higher values of the milk on each of the controls as on first and third milking controls the differences were statistically significant (P<0.05) (fig. 1). In our previous studies with ewes from the same flock similar values of the test day milk of first and sixth controls (1.1 l and 0,61 l) *(Ivanova and Raicheva, 2008a)* and of second control (0,862) *(Ivanova and Raicheva, 2008b)* were obtained.



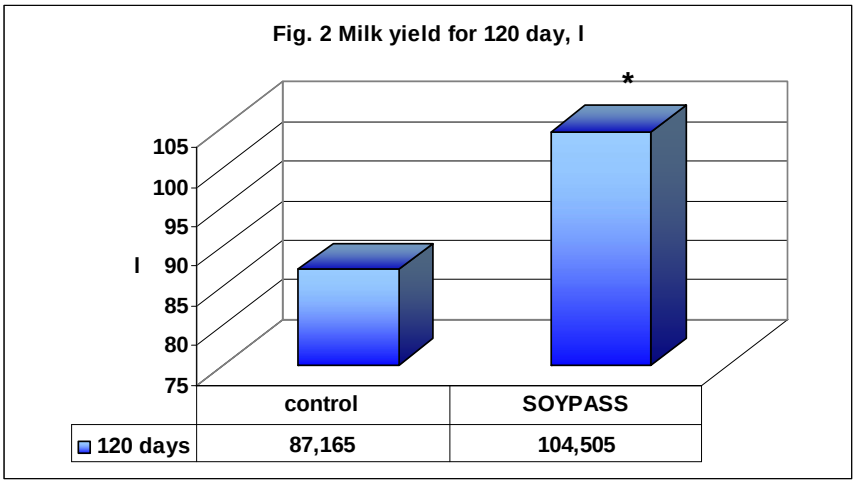
	I suc.	II suc.	III suc.	IV suc.	I	II	III	IV	V	VI	VII	VIII
control	2,37	1,83	1,27	1,19	0,969	0,88	0,752	0,75	0,706	0,625	0,564	0,565
SOYPASS	2,43	2,02	1,52	1,22	1,264	1,021	0,979	0,817	0,816	0,745	0,722	0,604

Note: Significant at: * - P<0.05

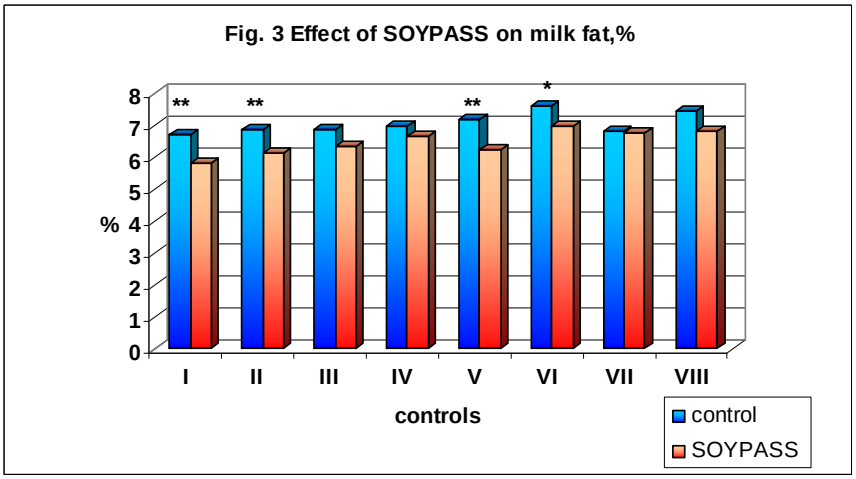
The standard milk production calculated for the 120 days milking period of the experimental ewes was characterized by significantly higher value compared to the controls (P<0.05) (Fig. 2). This result can be explained by the influence of SoyPass in the ration.

Previously in our experiments with sheep from the same flock similar value of the milk for 120 days milking period (90.75 l) was obtained *(Ivanova and Raicheva, 2010).*

The content of milk fats during the various lactation periods corresponds to the normal values for sheep milk (Fig. 3). In each of the controls we observed a tendency for higher fat content of the milk from the control group as it was statistically significant on the first, second, fifth and sixth controls (P<0.05; P<0.01).



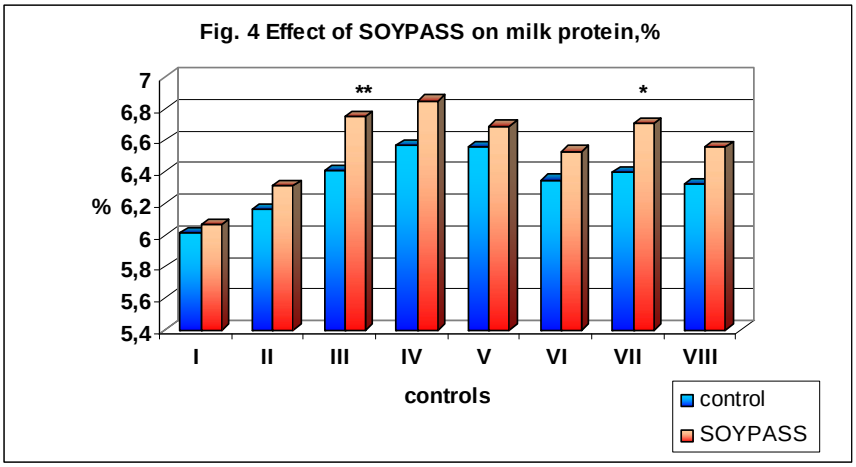
Note: Significant at: * - P<0.05



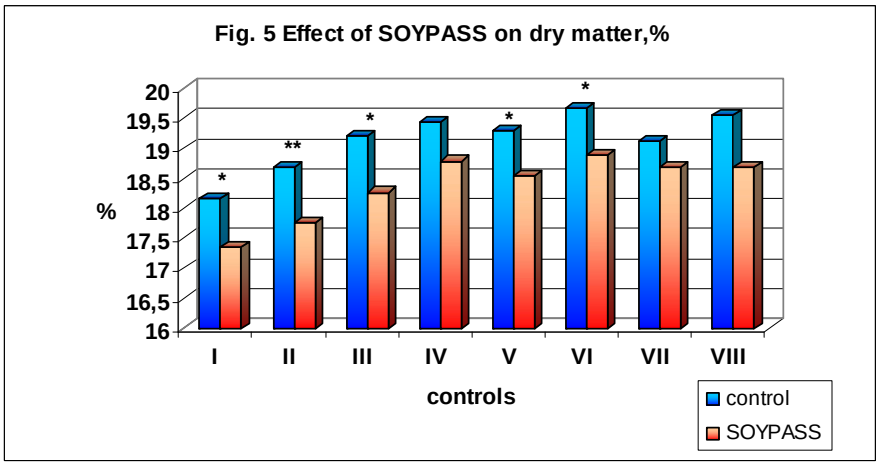
Note: Significant at: * - P<0.05; ** - P<0.01

We observed a tendency for higher protein content in the milk of the experimental group, statistically significant for the third and seventh controls (P<0.05; P<0.01) (fig. 4).

The dry matter contents during the various lactation periods showed specific trends for higher value in the control group as the differences were significant on the first, second, third, fifth and sixth controls (P<0.05; P<0.01) (fig. 5). Similar results for milk composition in ewes from Synthetic population Bulgarian milk were reported by *Raicheva et al. (2004)* and *Raicheva and Ivanova (2011)*.



Note: Significant at: * - P<0.05; ** - P<0.01



Note: Significant at: * - P<0.05; ** - P<0.01

The observed differences in the milk content – decrease of the fat and dry matter and increasing of protein content can be explained by the influence of

SoyPass. However the inclusion of SoyPass kept the fat, protein and dry matter percentages in the borders normal for the sheep milk.

Conclusion

The following conclusions could be made from the concrete study:

There is significant effect of SoyPass on the milk production for first and third milking controls ($P<0.05$) and for 120 days milking milk production (104.5 l) ($P<0.05$).

The values for the studied milk contents - milk fat and dry matter, were significantly higher in the control group ($P<0.05$; $P<0.01$).

The values for the protein content were significantly higher in the experimental group ($P<0.05$; $P<0.01$).

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Studija o uticaju Soypass na proizvodnju ovčijeg mleka

T. Ivanova, E. Raicheva

Rezime

Cilj studije je bio da se testira i oceni efekat zamene tradicionalne suncokretove sačme sa proizvodom SoyPass na proizvodnju ovčijeg mleka.. Eksperiment je izveden sa 40 ovaca sintetičke populacije bugarske mlečne rase iz zapata IAS - Kostinbrod, Bugarska. Trajanje eksperimenta je bilo 180 dana. Period adaptacije trajao je 15 dana do jagnjenja. Ovce su bile podeljene u dve grupe (eksperimentalna i kontrolna). Životinje iz obe grupe su izjednačene po uzrastu, broju laktacije, proizvodnji mleka i tipu jagnjenja. Ovce su hranjene u skladu sa standardima a potrošnja hrane je kontrolisana svakodnevno. Pored koncentrata, eksperimentalna grupa je dobila 7,5% SoiPass i 7,5% suncokretove sačme, a kontrola - 15% suncokretove sačme. Proizvodnja mleka je kontrolisana na svakih 15 dana. Denvna kontrola proizvodnje mleka – „the test day milk“ je utvrđeno prema AC metodi Icar-a. Sastav mleka tj. sadržaj mlečne masti, proteina i suve materije (%), određeni su na 20 ml uzorka mleka pomoću analizatora mleka Ecomilk, Bultech kompanije. Dobijeni podaci ocenjeni su varijaciono-statističkim metodama. Znatno veća proizvodnja mleka za standardni period muže od 120 dana koja je uočena u grupi eksperimentalnih životinja može se objasniti prisustvom SoyPass u obroku. Vrednosti za ispitivan sadržaj mleka - mlečne masti i suve materije, bile su značajno veće u kontrolnoj grupi ($p < 0,05$, $P < 0,01$). Vrednosti sadržaja proteina bile su značajno veće u eksperimentalnoj grupi ($p < 0,05$, $P < 0,01$).

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SOME FACTORS OF THE MAXIMUM TEST DAY MILK YIELD IN THE DAIRY POPULATION OF SHEEP IN MACEDONIA

N. Pacinovski[1] G. Dimov[2] E. Eftimova[1], N. Mateva[1], G. Cilev[3]

[1]UCAM Institute of Animal Science, Blvd Ilinden 92A, p. box. 207, 1000, Skopje, Republic of Macedonia,
[2]AgroBioInstitute, 8, Blvd Dragan Tsankov, 1164, Sofia, Republic of Bulgaria,
[3]Veterinary Faculty, Prilepska, BB, 7000, Bitola, Republic of Macedonia
Corresponding author: npacinovski@yahoo.com
Original scientific paper

Abstract: The test day milk yield (TDMY) was measured on 98 East Friesian ewes for 2 years period, 2005 and 2006. Totally 137 lactations were included. The daily yield was recorded twice a day, once monthly after birth of the lambs (A4 method). The weaning of the lambs was at about 60 days of age. The number test days (TD) varied from 5 to 11. Only the maximum of test daily milk yields (MTDMY) were analyzed for the effects of the year (Y), parity (P), number of lambs born (NL) and duration of the suckling period (SP). The period from lambing to the maximum test day yield was presented in days, as well as number of test day. Parity were from 1 to 6, number of lambs born from 1 to 3. The average period to the MTDMY was on the 50^{th}±3.1 day of the lactation and the main significant factor was the year ($P<0.001$). The parity and NL did not affect the period to the maximum TDMY. The average MTDMY was 1.83±0.04 l and was affected significantly by the parity ($P<0.001$). The maximum TDMY of 2.78 l was found for 4^{th} lactation which differed significantly from the others and the lowest of 1.72 l being 1^{st} and 6^{th}. In 73% of lactations the MTDMY appeared on the 1^{st} TD and in about 15% on the 3^{rd} and 4^{th} TD.A conclusion was made for the importance of the MTDMY in the improvement of the East Friesian breed of sheep in Macedonia.

Key words: maximum test day milk yield (MTDMY), East Friesian sheep, days to maximum milk yield

Introduction

Maximum test day milk yield (MTDMY) is an important trait in dairy sheep breeding as it reflects the potential of the ewes to produce milk. A number of

studies in the Mediterranean region examine the factors which affect the milk yield during separate test days (*Dimov et al., 2005, Djabirski et al., 2006, Gursoy et al., 1992, Pacinovski et al., 2010*) but no results were found for the maximum daily yield, e.g. for the peak of that yield.

The MTDMY is a trait of interest for selection because it reflects the total yield for the total lactation/milking only period. When the MTDMY is higher, also the total yield is higher. It is assumed that the MTDMY appears later for adult ewes and is higher compared to the 2.5 years old ewes (*Cappio-Borlino et al., 1997, Ruiz et al., 2000*).

The objective of the study was to determine the influence of different sources of variation on the maximum TD milk yield of the East Friesian ewes in Macedonia.

Material and Methods

The test day (TD) milk yield was measured on 98 imported East Friesian ewes for 2 years period – 2005 and 2006. Totally 137 lactations were included. The daily yield was recorded twice a day, once monthly with A4 method, (*ICAR, 2012*). For the whole lactations the number TD varied from 5 to 11. Only the maximum yields of these TD (MTDMY) were of interest for the study. The model included the fixed effects of the year (Y), parity (P) and number of lambs born (NL) as fixed class effects and the duration of the suckling period (SP), the period from lambing to the maximum TD milk yield as linear regressions. P were from 1 to 6, NL from 1 to 3.

The period from the lambing to the peak yield (number of days) was a complementary trait of interest.

The significance of the factors was estimated using F and T-tests (SPSS set of programs), (*SPSS, 1994*).

Results and Discussion

The average suckling period was 77.7±1.3 days and the period from birth to the peak value of test day milk yield was 49.9±2.6 days. The average maximum test day milk yield was 1.83±0.04 l (Table 1). The results showed that for the East Friesian (EF) ewes in Macedonia the maximum of a test day yield was in the beginning of the lactation period which is typical for majority of the studies of the lactation curve (*Cappio-Borlino et al., 1997; Kiss et al., 1997; Ruiz et al., 2000, Dimov, 1986*).

Table 1. Descriptive statistics data of investigated parameters

Trait	N	Min.	Max.	Mean
Suckling period, days	137	59	104	77.69±1.30
Day of peak value, days	137	8	175	49.88±2.60
MTDMY, lit.	137	0.9	4.0	1.83±0.04

The period to the lactation peak was affected significantly and considerably only by the year (Table 2). Lactation number, duration of the suckling period and number of lambs born did not affect the period to the maximum TD milk yield.

The MTDMY was affected mostly by the lactation number, while other factors were with no significant effect (Table 2).

Table 2. Factors influence on the maximum test day period (MTDP) and the maximum test day milk yield (MTDY)

Factor	Maximum TD period (MTDP)		Maximum TD milk yield (MTDMY)	
	df	F	Df	F
Year	1	15.6***	1	0.5ns
Lactation	4	1.1ns	4	9.2***
No of lambs born	2	2.9ns	2	0.8ns
Suckling period	1	0.1ns	1	1.1ns
Maximum TD period	/	/	1	0.5ns
R Squared =	/	0.402	/	0.423

*P<0.05, ** P<0.01, *** P<0.001, ns – non significant

In 2005 the period to the maximum yield (76,3 days) was twice longer (P<0.001), compared to 2006 (35,6 days), (Table 3). With the increase of the lactation number this period increased from almost 47-48 days for the youngest ewes (at 1st lactation) to 56 days at 4th lactation and 72 days at 5th lactation, with a consecutive decrease at 6th lactation. No one of these means however differed significantly. These figures differed to some extend from the studies of Awassi (*Kiss et al., 1997*) and Latxa in Spain (*Ruiz et al., 2000*), where the peak appeared almost at the same period for all lactations. A longer period with 8 days was found for the ewes with 2 lambs, compared to these with one lamb and the shortest period was found for the ewes with 3 lambs (Table 3).

Table 3. Estimates of the means for the effects of year, lactation and number of lambs born

Factor	N	Days to max MY, days	Max TDMY, l
Year			
2005	53	76.3±7.6 r	2.11±0.12
2006	84	35.6±6.2 r	2.00±0.09
Test Day			
1	98	36±8.0	1.88± 0.10
2	18	69±8.5	1.67±0.05
3	14	93±6.4	1.75±0.12
4	7	129±7.5	1.81±0.19
Lactaton			
1	61	47.8±6.1	1.72±0.09 re
2	62	48.7±6.0	1.98±0.08 sf
4	7	56.0±10.8	2.78±0.16 rsef
5	5	72.0±11.0	2.06±0.16 e
6	2	55.2±17.6	1.72±0.26 f
Number of lambs born			
1	71	58.2±6.2	1.96±0.09
2	58	66.4±6.3	1.99±0.09
3	8	44.3±10.4	2.21±0.15

a,b,c – $P<0.05$; e,f,g – $P<0.01$; r,s,t <0.001

The maximum TD yield for 2005 was 0.1 l higher than in 2006 ($P>0.05$), despite it was observed almost 40 days later in the lactation.

Higher MTDY was found for the later lactations with a maximum of as much as 2.78 l at the 4-th, compared to the earlier and later ones. It differed significantly from them and all the others did not differ between themselves. The results supported earlier findings for a higher MTDY for the later lactations (*Cappio-Borlino et al., 1997, Ruiz et al., 2000*).

The MTDY was slightly affected by the number of lambs born. The estimates of the MTDY showed a tendency for an increase of the MTDY from 1.96 l for the ewes with one lamb to 2.21 l for the ewes with 3 lambs. The differences however were not significant (Table 3).

The MTDMY was found at the 1st TD in 72% of lactations, and the later at 2-nd, 3-rd and 4-th TD in 13%, 10% and 5% of lactations, respectively. The result supports the findings of other authors for early appearance of the maximum yield. From breeding point of view the minor percentage groups are also of interest as they would be matter of choice for an increase of the yield during the milking only period when the maximum TD yield was in the later stages of the lactation.

Conclusion

The maximum test day milk yield in the imported East Friesian ewes in Macedonia was 1.8 l and was observed on the 50-th day of lactation.

The period to the maximum yield was affected by the year and the level of the yield by the lactation number.

In 28% of lactations the peak of the test day milk yield was found later, after the first test day and these ewes were considered to be of interest for the future selection.

Acknowledgment

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Neki parametri testa na maksimalnu dnevnu proizvodnju mleka kod mlečne populacije ovaca u Makedoniji

N. Pacinovski, G. Dimov, Elena Eftimova, Natasa Mateva, G. Cilev

Rezime

U radu je na osnovu vlastitih istraživanja određena dnevna mlečnost kod 98 ovaca istočno-frizijske rase. Ogled je trajao 2 godine (2005 i 2006). Uključeno je ukupno 137 laktacija i dnevna mlečnost je bila kontrolisana 2 puta dnevno, (ujutru i uveče), jednom mesečno, u periodu od jagnjenja do zasušenja. Jagnjad su odlučena u uzrastu od oko 60 dana. Broj test dnevne kontrole varirao je između 5 i 11. Maksimalna dnevna proizvodnja mleka u testu određena je pomoću efekta godine, uzrasta, broja novorođenih janjadi i dužine sisnog perioda. Period od jagnjenja do testa na maksimalnu dnevnu mlečnost, kao i dana u testu prikazani su u danima. Redosled laktacije bio je izmedju 1 i 6 a broj novorođene jagnjadi od 1 do 3. Prosečna maksimalna test dnevna mlečnost utvrđena je u 50±3.1 danu laktacije. Osnovni signifikantni faktor bila je godina ($P<0,001$). Redosled laktacije i broj novorođenih jagnjadi nisu uticali na period maksimalne dnevne proizvodnje mleka.

Prosečna maksimalna dnevna proizvodnja mleka u testu bila je 1,83±0,04 l, na koju je značajno uticao redosled laktacije ($P<0,001$). Maksimalna dnevna proizvodnja mleka u testu od 2,78 l bila je utvrđena u 4. laktaciji, koja se značajno razlikovala od ostalih, a najmanja od 1,72 l bila je utvđena u 1. i 6. laktaciji.

Kod 73% laktacija maksimalna dnevna proizvodnja mleka u testu utvđena je kod prve kontrole mlečnosti, a kod 15% u 3. i 4. kontroli.

Kao zaključak se moze navesti važnost obavljanja testa na maksimalnu dnevnu proizvodnju mleka za unapređenje istočno-frizijske rase ovaca u Republici Makedoniji.

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USAGE OF PALM OIL IN THE CHEESE PRODUCTION

G. Talevski

IMB "Mlekara" AD Bitola, Dolnoorizarski pat b.b. 7000, Macedonia
Corresponding author: g.talevski@t-home.mk
Original scientific paper

Abstract: The palm oil is a component, often added in the production of white brined cheeses in order to achieve high yield and getting a cheap product. Also it can be used as a replacement of milk fat in the season of milk shortage. The milk product produced by adding 12% of palm oil, after 30 days fermentation contains: 54.62% dry matter, 45.38% moisture, 44.31% fat, 82.40% fat in dry matter, 2.61% salt. The acidity is pH 4.03 and 75^{o} SH, the yield is amount up to 2.52 l/kg. The sensory analysis determines increased crumbliness and untypical rheological characteristics for brined cheese. The milk products with palm oil on the market are promoted as a dietary products, but are obligatory to be declared. The high content of trans fats can cause cardiovascular diseases in humans. The milk products with palm oil are recommended to be consumed in small quantities.

Key words: palm oil, white brined cheese, vegetable fats

Introduction

White brined cheeses are a distinctive group of cheeses that ripen in brine and their range of distribution is mainly in the Mediterranean countries, the Balkans, Eastern Europe, North Africa and some countries in Asia.

White brined cheese is a national dairy product in Republic of Macedonia with tradition long for centuries and best-selling cheese in the country. According to statistics for 2011 this type of cheeses are consumed in quantities of 7.4 kg per year, followed by 2.2 kg kashkaval cheese and urda (ricotta) with 2.1 kg by member of households *(Državen zavod za statistika, 2012).*

In recent years, caused by low standard, reduced purchasing power of the population and the desire for greater profit of dairy industry, on the market began a massive sale of milk products with vegetable fat, usually added palm oil. This can be increased by reducing the livestock in the country and the lack of raw milk for cheese production.

Palm oil is derived from tropical plant Elaeis guineensis and his frequent consumption increases the risk of coronary disease *(Crupkin and Zambelli, 2008).*

The use of palm oil dates back to 3.000 years BC and was found in a tomb at Abydos, and considered that Arab traders brought to Egypt *(Ndon, 2006)*.
This type of oil is widely used in the dairy industry, in the manufacture of ice cream, cakes, ready meals, confectionery etc. In the last period, palm oil consumption worldwide has increased from 1% to 15% of the total consumption of fat *(Reiner, 2000)*.

The high content of saturated fatty acids (palmitic and stearic) contributes to significantly increased levels of total cholesterol in humans as well as an increased ratio total / LDL cholesterol *(Mensink et al., 2003)*. In the production of cheese in which the raw material is milk, dominant fatty acid is the palmitic acid while the use of vegetable fats in addition of palmitic acid a high participation has oleic acid *(Dinkçi et al., 2011)*.

Palm oil with coconut oil is one of the few highly saturated vegetable fats. It is more firm at room temperature and has good characteristics for use in dairy products. According to *Andreasen and Nielsen (1998)*, palm oil contains palmitic acid C_{16} (45,5%), oleic C_{18} (39%), linolenic $C_{18:2}$ (9%), stearic C_{18} (5%), miristic C_{14} (1%) and arachidonic C_{20} (0,5%). Iodine number of palm oil by the authors is 51.5 while the milk fat 34.5.

According to some authors palm oil if is used in moderation has positive effects on human health. So determined is that it accelerates the process of wound healing, has antimicrobial properties, has great energy potential, contains vitamins like A, E, beta carotene etc. *(Ekwenye and Ijeomah, 2005; Mukherjee and Mitra, 2009)*.

According to *Noronha et al. (2008)*, the imitations or cheese analogues are defined as products like cheese made by mixing the individual ingredients including no milk fat or protein to form a cohesive mass similar to cheese. Although some dairies represent cheeses with vegetable fats as dietary products yet consumers are becoming increasingly aware of the relationship between diet and health. EU with adoption of Regulation (EC) 1324/2007 indicates that the replacement of milk with plant components, the final product can`t be called cheese, butter or milk.

The purpose of this paper is to present chemical and physical properties of the prepared mixture for the production of milk product by adding palm oil, whey obtained during the production and chemical composition of the milk product. Also are presented the most important sensory properties of produced milk product with palm oil.

Material and Methods

Experimental part in the production of milk product by adding 12% palm oil was performed in dairy "Bistra"-Kičevo in 7 repetitions. This product is made from 50% whole milk and 50% mixture consisting of milk powder, water, palm oil and emulsifier (distilled monoglyceride). Melting of palm oil is performed at a temperature of 40 ºC, mixed with other ingredients of the mixture is homogenized at 110 bar/65 ºC and pasteurized at 82 ºC/15 min.

Pasteurized mixture is cooled to 41 ºC, $CaCl_2$ (0.002%) is added, DVS culture YC 331 composed of bacteria: *Lactobacillus delbrueckii sp. bulgaricus* and *Streptococcus thermophilus* and coagulation with rennet powder Chymax powder extra. Coagulation is performed for 45 minutes, followed by processing of coagulum, pressing with 2 kg / 1 kg on curd for 2 hours, cutting the obtained curd into pieces and ripening. Brining is performed 16-18 hours in brine measuring 16-18 ºBe, pH 4.0-4.2; brine temperature of 16-18 ºC. After brining is following packaging of the product in the containers, pouring the brine with 8 ºBe salt and pH 2.6-2.8. Ripening in the chamber with a temperature of 16-18 ºC to achieve pH 4.0-4.2 for several days, after which the milk product with 12% palm oil can be placed in the retail network or storage at 4 ºC.

Chemical analysis of the mixture for production of this type of milk product were conducted in internal laboratory of the dairy with the device Ekomilk and it was determined the presence of protein, lactose, fat, dry matter and non-fat dry matter. The quantities of these compounds were measured in the whey from production of the milk product.

Chemical analyses of the milk product with 12% palm oil were performed at the Institute of Food at Faculty of Veterinary Medicine - Skopje. To perform the analysis in this product were applied following methods: dry matter (the reference method by drying at 102 ± 1 ºC), fat (by the method of Van Gulik), salt (according to the method described by *Carić et al., 2000)*, active acidity-pH (using a pH-meter Mettler Toledo), titratable acidity (by the method of Sokshlet-Henkel). The contents of moisture, fat in dry matter and salt in aqueous phase were calculated. Statistical analysis of data was carried out using the Microsoft Excel program that is part of the suite Microsoft Office.

Results and Discussion

Table 1 shows the chemical composition of the mixture for the production of milk product with 12% palm oil. From the data it can be seen that the average dry matter content of 20.22% is approximate as sheep milk according *Pavičić*

(2006), contains about 19.01% dry matter and 80.99% water. The largest share in the dry matter of the mixture have fat ($\bar{x}$=11.67%), which represented 57.71% of the total dry matter content. The high percentage of fat in the mixture comes from adding palm oil during processing remains in the product and contributes to a higher yield over the cheese produced by the usual technological process.

Table 1.Chemical and physical properties of the mixture for the production of milk product with palm oil (n=7)

Parameter	$\bar{x}$	min	max	SD	CV
Dry matter	20.22	19.14	21.14	0.6835	3.38
Non-fat dry matter	8.55	8.34	8.78	0.1844	2.16
Fats	11.67	10.80	12.50	0.5931	5.08
Proteins	3.40	3.31	3.51	0.0724	2.13
Lactose	4.42	4.38	4.50	0.0404	0.91
pH	6.67	6.66	6.68	0.0069	0.10
^{0}SH	6.34	6.2	6.4	0.0976	1.54

Full-cream cow's milk contains 3.7% fat milk *(Muir, 1998)* while in our testing fat content ranged from 10.80 to 12.50%.

The active acidity of pH 6.67 and titratable acidity with 6.34 ºSH was within the normal range for milk used in cheese production.

Table 2 shows the chemical composition of the whey which is obtained as a byproduct in the production of milk product with 12% palm oil. From the table it can be seen that the percentage of fat is very low 0.02% due to their incorporation into the curd. Palm oil melts at 36-40°C and further technological operations in the manufacture of this kind of milk product comes to cooling the curd, fat hardens and stays in the curd.

Fermentation of the milk product with palm oil takes place on 16-18°C, while storage is at 4 °C, so there is no opportunity to take palm oil in brine but it remains incorporated within the curd. The effect of palm oil in large measure prevents losses, because at cow milk cheese for 30 days losses around 10-12% until milk product with vegetable fat only 2-4%.

Table 2.Chemical and physical properties of the whey (n=7)

Parameter	$\bar{x}$	min	max	SD	CV
Dry matter	7.72	7.58	7.93	0.1077	1.39
Non-fat dry matter	7.70	7.53	7.88	0.1101	1.43
Fats	0.02	0	0.05	0.0267	133.5
Proteins	0.42	0.31	0.52	0.0379	9.02
Lactose	6.63	6.41	6.80	0.1396	2.10
pH	5.90	5.84	5.97	0.0434	0.74
^{0}SH	7.00	6.80	7.20	0.1633	2.33

The higher amount of lactose in the whey was due to the addition of skimmed milk powder in the mixture for this product. Milk powder is rich in lactose and because much of it is eliminated with the whey. Data from the table can be determined that the average content of lactose was 6.63% with a minimum 6.41% and maximum 6.80%. According *Hramcov (1979)* whey contains approximately 4.5% lactose, 0.7% protein and 0.4% fat. Whey protein level in our tests is lower because using high temperature of pasteurization most of the whey and casein proteins are retained in the curd. pH of the whey was 5.90 while titratable acidity 7.0 ºSH was due to intense acidification of the curd in this type of product.

Table 3 presents the chemical composition of the milk product by adding 12% palm oil. Dry matter tends to increase during the test period and 49.16% (1 day), increases of 52.26% (10th day) and reached 54.62% (30th day).

Table 3. Chemical composition of the milk product with 12% palm oil (n=7)

Parameter	1 day			10 days			30 days		
	$\bar{x}$	SD	CV	$\bar{x}$	SD	CV	$\bar{x}$	SD	CV
Dry matter	49.16	3.0765	6.26	52.26	2.5686	4.91	54.62	2.3554	4.31
Moisture	50.84	3.0765	6.05	47.93	2.7443	5.73	45.38	2.3554	5.19
Fats	40.36	1.5469	3.83	42.63	2.4736	5.80	44.31	1.8279	4.13
Fat in dry matter	82.17	3.1568	3.84	81.59	2.5757	3.16	82.40	1.5603	1.89
Salt	0	0	0	1.93	0.2289	11.87	2.61	0.1069	4.09
Salt in water	0	0	0	4.02	0.5359	13.28	5.77	0.3722	6.45
pH	4.42	0.2222	5.03	4.05	0.0951	2.35	4.03	0.0912	2.26
^{0}SH	52.87	6.4711	12.24	65.86	4.3370	6.59	75	4.2383	5.65

The increasing of dry matter in the product is caused by applied citric acid in the brine which contributes to greater migration of water from the curd and getting a solid and compact pieces. Also with technological operations is strive the curd grains to stick and to speed up syneresis from cheese curd, while is still hot and pressing must be quick because this type of product for its consistency and structure are classified between pressed ricotta and typical white brined cheese. Any delay in technological processes contributing to get soft, crumbly product with unconnected curd grains which decays at least manipulate it.

Moisture after 30 days fermentation was 45.38%, which is lower compared to cow cheese produced from cow's milk which according *Baltadjieva and Slavchev (2003)* is between 52-54%. The content of fat in this type of milk product amounted to 44.31% due to the additional input of palm oil in the milk. Palm oil reduces costs in the production of cheese, is quite cheap and are incorporated into the cheese curd as charge giving good sensory properties, good texture and consistency.

These values are far more fat if you compare the content of fat in several Balkan brined cheeses where according *Dozet and Maćej (2006)* range from 25 to 27.93%. The same can be noted for fat in dry matter which had enormous values of 82.40% after 30 days fermentation.That percentage is far higher in terms of fat content in dry matter in different types of white cheese studied by numerous authors where values in the interval ranged from 32.90% to 64.80% *(Dozet et al., 1996)*.

The results for the salt content showed us an increase of 1.93% (10 day) to 2.61% (30 day). Palm oil has some impact on osmotic-diffusion processes of salt in cheese, because it hardens at the temperature of fermentation and slower the penetration of salt in cheese. The ratio of salt in the aqueous phase is an important parameter for monitoring the activity of lactic acid bacteria.

From the table it can be seen that the salt in the aqueous phase increases from 4.02% on the 10th day of 5.77% on the 30th day. That indicates although milk product with palm oil is designed for fast implementation, however fermentation should last at least a month. These data coincide with the conclusion of *Kožev (2006)* which states that lactic acid bacteria at concentrations of salt in the aqueous phase of 1-1.5% develop normally, at a concentration of 2-3% are noted some slowdown in growth, while over 6.5% completely stop their growth.

By reducing the active acidity from pH 4.42 on the first day, to 4.05 on the tenth day until reaching 4.03 units on the 30th day it can be concluded that stormy fermentation takes place in the first ten days. Similarly there is increase in the acidity of titratable 52.87 ºSH (1 day) to 75 ºSH (30th day). The reduction of the active acidity and its influence has added citric acid used in the preparation of brine.

From our observations we can conclude that milk product with palm oil must been ferment at least a month until reaching approximately pH 4.0 to obtain quality packaging in the polymer foil. If the milk product with palm oil is packaged at higher pH (4.2 to 4.5) which is characteristic of the normal white brined cheese often comes to the accumulation of gases from metabolic processes of transformation of lactose into lactic acid and the cheese packaging is devacuumed. Full conversion of lactose into lactic acid is extended in this type of milk product that probably has an impact palm oil and higher content of lactose, which is located in added milk powder in a large percentage are incorporated in the cheese curd. Therefore an extended period of transformation into lactic acid which indicate *Fayed et al. (1988)*.

Often dairies do not declare the addition of palm oil in the production of cheese, or used as a general term vegetable oil or vegetable fat. That has confirmed *Naydenova et al. (2010)* in their investigations. Some dairies to achieve higher profits often make marketing tricks declaration of such cheeses as "light" products.

But with the latest EU legislation starting from 2015, all food manufacturers are required to declare and specific type of vegetable oil that has added to the product (e.g. coconut oil, palm oil, soybean oil) *(USDA, 2012)*. Trans fats are present in large amounts in the diet in the countries of Northern Europe to the Mediterranean countries often use olive oil.

According to *Filip et al. (2010)*, the daily intake of trans fats in Greece is 1.4 g while in Iceland 5.4 g. In this context *Pin Koh and Wilcove (2007)* point out that palm oil can be considered a replacement of trans fats, but other authors as *Vega Lopez et al. (2006)* noted that palm oil causes adverse changes in the concentration of LDL cholesterol and apolipo-protein B levels as well as trans fats. That fact is confirmed by WHO which states that palmitic acid is dominant fatty acid in palm oil increases the risk of cardiovascular disease and are classified in the same category as trans fatty acids *(WHO, 2003)*.

Rheological assessment of the milk product with palm oil has shown that it has little elasticity in the external shock or pressure and greater crumbliness compared to standard white brined cheese.

The first period of fermentation is characteristic with smell of palm oil, but later the smell is disguised with the citric acid, and the increasing acidification of the cheese curd. These characteristics are noted by *Fayed et al. (1988)* which indicate that the samples of cheese Domiati which is from 0 to 50% palm oil has better sensory properties than those samples which contains 75-100% palm oil. The milk product with a higher content of palm oil felt buttery "off flavour" smell, that is lost after 8 weeks of fermentation. Yield of milk product with 12% palm oil is 2.52 liters of a mixture for 1 kg of product, while for producing of 1 kg standard white brined cheese by *Chomakov et al. (2000)*, requires 6.75 to 6.89 liters of cow milk.

The general conclusion is that milk product with palm oil can be consumed in small quantities because the chemical composition, nutritional and sensory properties deviate from standard white brined cheese and can cause adverse health effects in the population.

Conclusion

The milk product by adding palm oil is an inexpensive product designed for quick consumption and characteristic of countries with a shortage of raw milk and the low purchasing power of the population. The chemical composition of this type of milk product is characterized by a high percentage of fat that can cause cardiovascular diseases in humans. The milk product with palm oil in sensory analysis is characterized by increased crumbly structure which falls between standard white brined cheese and pressed ricotta. Obligatory declaration of the type

of added vegetable fat in cheese is important for health and nutritional aspects of the population.

Primena palminog ulja u proizvodnji sira

G. Talevski

Rezime

Palmino ulje je komponenta koja se često dodaje u proizvodnji belih salamurenih sireva sa ciljem postizanja visokog randmana i dobijanja jeftinijeg proizvoda. Takođe se koristi kao zamena za mlečne masti u sezoni nestašice mleka.

Mlečni proizvod sa dodavanjem 12% palminog ulja, nakon 30 dana fermentacije je sadržao: 54.62% suve materije, 45.38% vlage, 44.31% masti, 82.40% masti u suvoj materiji, 2.61% so. Kiselost je iznosila pH 4.03 i 75 ^{0}SH, randman je bio 2.52 l/kg.

Senzorna analiza je pokazala povećanu trošljivost i netipične reološke karakteristike za beli sir.

Mlečni proizvodi sa palminim uljem u prodaji se promovišu kao dijetalni proizvodi, ali obavezno moraju biti deklarisani. Visok sadržaj trans masti može da izazove kardiovaskularna oboljenja kod ljudi. Mlečne proizvode sa palminim uljem preporučljivo je konzumirati u malim količinama.

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COMPARATIVE STUDY OF FERTILITY IN A STANDARD MATING PROCEDURE AND AFTER HORMONAL TREATMENT TO INDUCE OESTRUS AND OVULATION

P. Slavova, S. Laleva, Y. Popova

Agricultural institute – Stara Zagora, 6000 Stara Zagora, Bulgaria
Corresponding author: pkslavova@abv.bg
Original Scientific paper

Abstract: The purpose of this study was to analyze the fertility level during the season as standard and fertility after application of hormones to induce oestrus and ovulation. The experiment was carried out with two groups of sheep of Trakian merino breed in the sheep farm of Agricultural Institute - Stara Zagora. The first group counted 96 animals treated with hormones /according to the adopted scheme/ out-of-season - in May. The second group - 112 animals were inseminated in July-August as regular. In our study we used the following scheme of hormonal treatment: putting vaginal sponges for sheep type Sincro-part / 30mg /, removing them in 12 days and giving sheep an injection of PMSG at a dose of 500 UI and applying artificial insemination at the 50-55th hour after injection. When the lambing season came to its end the final results were summarized, analyzed and calculated. It was found that: the difference in the proportion of aborted sheep and lambed sheep as well as the proportion of live births and stillborn lambs from both groups was insignificant. The received off-springs are doing well; biological and economic fertility is significantly higher in sheep treated with hormones - the difference is respectively 42.97% and 12.55%.; applying the testing hormonal scheme results in a greater number of lambs, higher economic effect and is relevant to the good management system of sheep production in our country.

Key words: Sheep, reproduction**,** hormonal treatment, vaginal sponges.

Introduction

Fertility level is a major selection indicator of great economic importance. From 60% to 80% of income in sheep production /in different production trends/ comes from lambs to the market. Intensification of breeding process and induction of oestrus out-of-season can be achieved by application of various hormones.

To make a greater economic effect of sheep production it is very important to increase number of lambing per year as well and optimize individual processes in order to have larger lamb production for the market and for breeding. This is possible if reproductive cycle in sheep is managed well by inducing oestrus and superovulation using vaginal sponges.

Synchronization of estrus allows obtaining equal groups of lambs so they can be grouped by age and allows also their early weaning and good nutrition, as well as artificial insemination to apply. This is particularly important for pure-bred stud farms where we need to keep up a high level of selection.

The usage of vaginal sponges is well known in practice in recent years and applied by many researchers in different sheep breeds *(Boscos et al., 2002; Jafar Yadi et al., 2011; Osama et al., 2010; Ralchev et al., 2008).*

Several authors have examined the effects of different doses of gonadotropic preparations to obtain the best effect of their application *(Ralchev et al., 2008; Naqvi et al., 2011; Quintero-Elisea et al., 2011).*

In our previous studies *(Bonev et al., 2001, Bonev et al., 2002, Bonev et al., 2004)* we tested different schemes for hormonal induction of oestrus out-of season in Ile de France and Trakian merino breed. The object of the study was to optimize the PMSG dosage, as well as to reveal the interdependence between maternal age and PMSG dosage and born lambs.

The purpose of this study was to analyze the fertility level during the season as standard and fertility after application of hormones to induce oestrus and ovulation.

Material and Methods

The experiment was carried out with two groups of sheep of Trakian merino breed in the sheep farm of Agricultural Institute - Stara Zagora. The first group counted 96 animals treated with hormones / according to the adopted scheme / out-of-season - in May. The second group - 112 animals were inseminated in July-August as regular. In our study we used the following scheme of hormonal treatment: putting tampons for sheep type Sincro-part / 30mg /, removing them in 12 days and giving sheep an injection of PMSG at a dose of 500 UI and applying artificial insemination at the 50-55th hour after injection. When the lambing season came to its end the final results were summarized, analyzed and calculated.

Results and Discussion

In Table. 1 the results of mating and lambing in sheep are shown. From the animals which had been treated with hormones in Group I - 72.92% were mated and 27.06% remained not mated. A smaller number of sheep remained not mated in Group

II - 16.42%. The difference in the proportion of aborted and lambed animals from both groups was insignificant - respectively 97.14% and 2.86% versus 97.32% and 2.68%.

Table 1. Mated ewes

Traits	I group		II group	
	number	%	number	%
Treated and inseminated	96	100	134	100
Mated	70	72,92	112	83,58
Unmated	26	27,08	22	16,42
Sheep with lambs	68	97,14	109	97,32
Abortion	2	2,86	3	2,68

The results for live births and stillborn in both groups /Table.2/ are similar. There are very significant differences between biological fertility - 42.97% and economic fertility - 12.55%. These results correspond with those of *Boscos et al. (2002)* and have been expressed in the larger number of lambs born per ewe.

Table 2. Fertility

Traits	I group		II group	
	n	%	n	%
Lambs received	111	100	130	100
Live births	107	96,40	126	96,92
Stillborn births	4	3,60	4	3,08
Biological fertility	-	163,24	-	119,27
Economic fertility	-	115,63	-	103,08

Based on the results obtained may be the finding that treated sheep /despite having lower number of sheep mated / have significantly higher fertility so that we receive a larger number of lambs from ewe and greater economic effect. Another advantage of the hormonal scheme applied is receiving lambs in such a period of the year that allows putting them on the market during major national holidays at a higher price than usual. The remaining sheep not mated during the out-of-season breeding period can be mated during the seasonal breeding campaign. Costs made for the organization and execution of events are small - 3.65 euros per sheep and pay -off is several times bigger because of the larger number of lambs received.

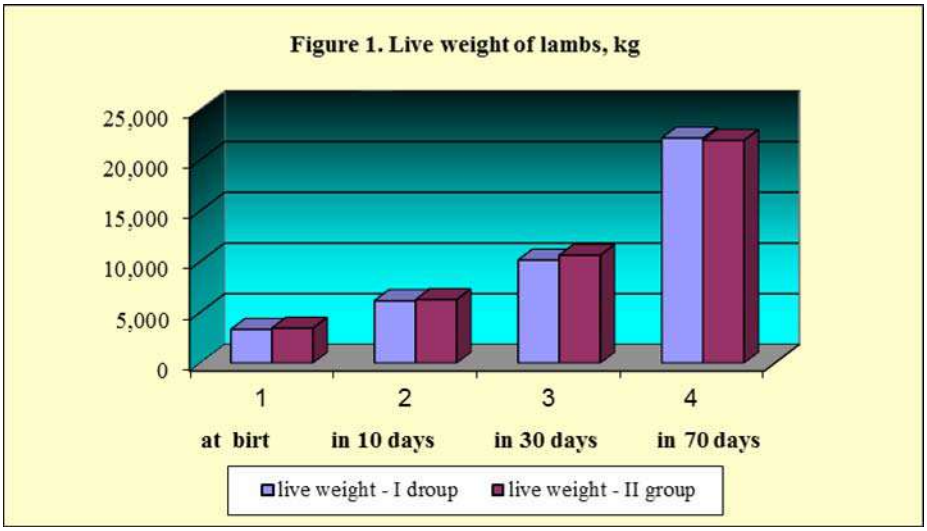
Very important is the question of the vitality and development of the newborn lambs as a result of the application of this scheme of hormonal treatment / Table. 3 /.

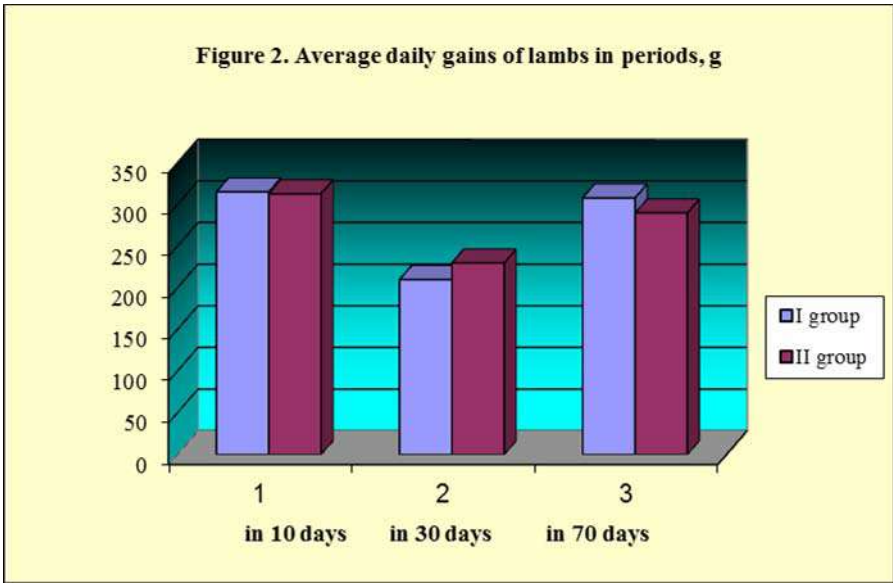
Table 3. Live weight of lambs

Age	I group			II group		
	N	x ± Sx	C	N	x ± Sx	C
At birth	107	3,311 ± 0,083	25,84	126	3,444 ± 0,068	22,21
In 10 days	106	6,149 ± 0,156	26,15	121	6,257 ± 0,109	19,13
In 30 days	103	10,148 ± 0,240	23,43	121	10,630 ± 0,210	21,69
In 70 days	92	22,177 ± 0,373	16,15	119	21,947 ± 0,314	15,59

Their live weight at birth and 10 days after birth did not differ significantly from that of lambs from the second group; at the 30th day the difference is 482 g. The results are reasonable considering the large number of sheep having twins, which inevitably affects the weight of the newborn. At 70 days of age the difference was 230 g in favor of the lambs from the first group (Fig. 1), which indicates that they compensate for growth thanks to nutrition. Average daily gain by periods is represented on Figure 2. Reducing the number of animals with the age grows up is due to dropping out or sale.

Figure 1. Live weight of lambs, kg





First group
Dropouts - up to 10 days / 1 crushed /
- to 30 days / 1 crushed and 2 sold /
- to 70 days / 1 dead, 1 crushed and 9 sold /
Second group
Dropouts – up to 10 days / 4 died and 1 sold /
- 70 days / 2 sold/

Conclusion

- The difference in the proportion of aborted sheep and lambed sheep as well as the proportion of live births and stillborn lambs from both groups was insignificant. The received off-springs are doing well.
- Biological and economic fertility is significantly higher in sheep treated with hormones - the difference is respectively 42.97% and 12.55%.
- Applying the testing hormonal scheme results in a greater number of lambs, higher economic effect and is relevant to the good management system of sheep production in our country.

Komparativna studija plodnosti standardne procedure parenja i nakon hormonskog tretmana indukcije ovulacije i estrusa

P. Slavova, S. Laleva, Y. Popova

Rezime

Cilj ove studije bio je da se analizira nivo fertiliteta tokom sezone, kao standarda i plodnosti nakon primene hormona u indukciji estrusa i ovulacije. Eksperiment je sproveden sa dve grupe ovaca rase trakijski merino na ovčarskoj farmi Poljoprivrednog instituta - Stara Zagora. Prva grupa broji 96 životinja tretiranih hormonima / prema usvojenom planu / van sezone - u maju. Druga grupa - 112 životinja su osemenjene u julu i avgustu po redovnoj proceduri. U našoj studiji smo koristili sledeću šemu hormonske terapije: stavljanje vaginalnih sunđera za ovce tipa Sincro-part / 30mg /, koji se uklanjaju nakon 12 dana, a ovce dobijaju injekciju PMSG u dozi od 500 UI i veštački se oplođuju 50. -55. sati nakon ubrizgavanja. Po završetku sezone jagnjenja konačni rezultati su sumirani, analizirani i izračunati. Utvrđeno je da: razlika u broju abortusa i ovaca koje su se jagnjile, kao i udeli živorođenih i mrtvorođenih jagnjadi iz obe grupe nije bio signifikantan. Dobijeno potomstvo je dobro; biološka i ekonomska plodnost je znatno veća kod ovaca tretiranih sa hormonima - razlika je 42,97%, odnosno 12,55%, a primena hormonske šeme rezultirala je u većem broju jagnjadi, većem ekonomskom efektu i od značaja je za dobar sistem upravljanja ovčarskom proizvodnjom u našoj zemlji.

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EFFECT OF DRY DISTILLER' GRAINS WITH SOLUBLES FROM CORN (DDGSc) ON SOME WETHER` BIOCHEMICAL PARAMETERS

M. R. Yossifov, L. K. Kozelov, M. A. Petkova

[1]Institute of Animal Science Kostinbrod 2232, Bulgaria
Corresponding author: m_vet@abv.bg
Original scientific paper

Abstract: Recently, the new technologies for ethanol production lead to an enhanced role of this industry and the production of feed by-products (dried distiller' grains (DDG), gluten meal, etc.). The shortage and continuous price enhancement of traditional feedstuffs for animals throw out a challenge for searching their alternatives. Investigations in this area are insufficiently in Bulgaria. It's interesting to study our own Bulgarian dry distiller' grains with soluble from corn (DDGSc) as a component of diets for small ruminants. The objective of our study was to determine the effects of DDGSc on wethers' blood biochemical changes. Two rations (Control Ration, CR and Experimental Ration, ER) were made. They contained 63 % compound feeds and 37 % roughage. The main components of both compound feeds were corn and tritikale. The experimental ration contained DDGSc as substitute of sunflower meal from Control ration. Both diets were equivalent on protein contents. The chemical composition of rations was determined by the conventional Weende analysis. The following blood biochemical parameters (before feeding and 2.5 h after feeding) have been studied: total protein, albumin, globulin, urea, cholesterol, triglycerides, and both AsAT and AlAT enzymes` activities. All biochemical parameters were estimated by Pentra 400 automatic biochemical analyzer. The obtained results showed that there were no any incidences of digestive disorders in both investigated groups. It's concluded that our choice of DDGSc as alternative nutritive component of ration for small ruminants and as a substitute of sunflower meal had been suitable. On the base of these results we could go on to evaluate DDGSc effect on rumen degradability, digestion in the whole tract, utilization of nutrients, animal production (meat and milk) for both quality and quantity.

Key words: feedstuffs, distillers dried grains with soluble from corn (DDGSc), composition, nutritive value, wethers, blood biochemical parameters

Introduction

Rapidly increasement in the number of distilleries for bioethanol production result in enormous quantities of available by-products suited for animal diets. So, this group of bioethanol subproducts very often are used in ruminant diets as protein supplement (especally in finishing feedlot diets). However, the sheep-breeding sector accepted furtively these untraditional and nonconventional feedstuffs as a source of protein and energy at total rations (TR). One of the main reasons is relatively poor researching data and information with practical usage about dried distiller's corn grains with soluble (DDGSc) in small ruminant's diets. Amassing knowledge for DDGS' processing, chemical composition, animal performance, etc. must be lined by evaluation of series of biochemical parameters, such determining animal performance.

Recommended DDGS levels included in sheep diets varied up to 10 – 15 % (*www.wcfin.ca.pdf*), in spite of lacking information about optimal including levels. DDGSc contain both rumen degradable protein (RDP) (up to 60 % of the CP) and fat (12 %). Low content of starch (approximately 70 % of the weight of grain) and availability of physilogical active fibers (primarily nonstructural carbohydrates) changed slowly rumen pH, resp. reduced the possibility of rumen acidosis (moderate rumen pH fluctuations) (*Fluharty et al., 1994*).

The objectives of our trial was to evaluate the effects of DDGSc (high fat and low fibres) as a substitute of sunflower meal (SFM) (low fat and high fibres) on some blood biochemical parameters.

Material and Methods

Animals and rations. In the experimental base of the Institute of Animal Science (IAS) Kostinbrod, BG was conducted an experiment with four wethers aligned by age (4 years), breed (Cygay) and body weight (mean 63 ± 2 kg). Animals were kept in metabolic cages with free access to water. The experimental period was performed using the preliminary (7-d) and experimental (4-d) period. The experimental design is shown in table 1. Total rations (TR) were composed of roughage (meadow hay - MH) and concentrate mix. The cereal-based concentrate mix entered corn grain and triticale. The third component, protein source, in the control diet (CD) was sunflower meal (SFM), and in the experimental diet (ED) - dried distiller' corn grains with soluble (DDGSc). Rations were formulated to be

Table 1. Experimental diets (g) and (%)

Diet	Feedstuffs	Control diet (CD)		Experimental diet (ED)	
		(g)	(%)	(g)	(%)
Concentrate mix	Corn	321.54	17.65	128.61	6.91
	Tritikale	321.54	17.65	321.54	17.28
	Protein source*	493.02	27.05	724.54	38.94
	Total	1136.10	62.35	1174.69	63.13
Roughage	Meadow hay	685.95	37.65	685.95	36.87
Total ration	Total	1822.05	100	1860.64	100
* Sunflower meal (SFM) for CD and dried distiller's corn grains with soluble (DDGS) for ED					

iso-caloric (1.1 FUG), iso-nitrogenous (16 %) and equal in Ca:P ratio (2.0), meeting or exceed actual requirements (*NRC, 2007*). The feeding was individual, twice a day with equal quantities. Diets were limit fed and consisted of similar amounts MH (37 %), cereal component (35 % in CD and 24 % in ED) and either SFM (27 %) or DDGSc (39 %).

Experimental procedures. Experiments were 14 days – 10-d preparatory + 4-d experimental period. Blood samples were collected by jugular venipuncture into 4 mL Vacutainer tubes with *Clot*- activator from each individual animal before morning diet at 8.00 h and 2.5 h post feeding. Blood samples were taken twice, on 1-d (11) and 3-d (13). Tubes were centrifuged at 1,500 g at room temperature for 10 min. The following biochemical parameters were studied: total protein, albumin, globulin, urea, cholesterol, triglycerides, and enzyme activity of AsAT and AlAT aminotransferases.

Chemical analyzes, calculations and statistical methods. Chemical composition of feedstuffs and diets were determined by conventional Weende analysis (*AOAC, 2002*). Automatic biochemical analyzer was used for blood test. The results have been statistically analyzed and interpreted by computer software for statistical analysis - *Microsoft Excel 2007*, a single factor analysis using ANOVA program. Values are expressed as mean ± SD. Tables, figures and graphics were made of the same software.

Results and Discussion

Diet composition and consumption. Chemical composition of feedstuffs is shown in table 2.

Table 2. Chemical composition of diets components

Feedstuffs	DM %	CP*	EE*	CF*	Ash*
Corn	81.66	6.03	2.21	2.55	0.85
Tritikale	83.57	11.60	1.20	3.11	1.55
SFM	86.32	32.15	0.72	18.00	6.47
DDGSc	87.42	23.08	10.02	6.72	3.84
Meadow hay	84.74	7.18	0.92	30.31	4.91

* as fed basis
DM-dry matter; CP-crude protein; EE-ether extract; CF-crude fibres; Sunflower meal-SFM; Dried distiller's corn grains with soluble-DDGS

Including 38 % DDGSc in sheep' TR as a substitute of 27 % SFM + 11 % cereal component did not affected significantly animal comsumption (table 3). Received actual values for dry matter intake (DMI) (1538 vs. 1588 g), crude protein (264.45 vs. 261.53 g) and ash (73.29 vs. 67.57 g) are similar in both groups (+ 3.1, - 1.1 and - 7.8 %). CF consumption (234.98 vs. 269.88 g) varied 15 % and EE (20.83 vs. 85.61 g) exceeded tripple CD, meeting the objectives of our study.

Biochemical parameters. Serum metabolites are frequently used to monitor the metabolitic health status of productive animals (*Ametaj et al., 2009*). Therefore, it should be noted that all values are obtained within the reference for this species. This applies to both - groups and dynamics. The influence of the ED on blood biochemical changes is presented in table 4.

Table 3. Consumption of forage, DM and nutrients from concentrate mixture, roughage and total ration, among the groups (g)

	Diet	CD	ED
As fed basis (g)	Concentrate mix	1136.10	1174.69
	Roughage	685.95	685.95
	Total ration	1822.05	1860.64
DMI (g)	Concentrate mix	956.95	1007.12
	Roughage	581.27	581.27
	Total ration	1538.22	1588.39
CP (g)	Concentrate mix	215.20	212.28
	Roughage	49.25	49.25
	Total ration	264.45	261.53
EE (g)	Concentrate mix	14.52	79.30
	Roughage	6.31	6.31
	Total ration	20.83	85.61
CF (g)	Concentrate mix	27.07	61.97
	Roughage	207.91	207.91
	Total ration	234.98	269.88
Ash (g)	Concentrate mix	39.61	33.89
	Roughage	33.68	33.68
	Total ration	73.29	67.57

DMI-dry matter intake; CP-crude protein; EE-ether extract; CF-crude fibres; Sunflower meal-SFM; Dried distiller's corn grains with soluble-DDGS; CD-Control diet; ED-Experimental diet

DDGSc inclusion showed no significantly affect on total protein (TP), albumin (Alb), globulin (Glob), urea (BUN), cholesterol (Chol), triglycerides (Trigly), aspartate (AsAT) and alanine (AlAT) aminotransferases concentration ($p>0.05$).

Table 4. Effect of DDGS supplementation on some blood biochemical parameters (n=8; x±SD)

Indicators		Control diet		Experimental diet	
		0.0 h	2.5 h post	0.0 h	2.5h post
TP	$g.dL^{-1}$	6.73 ± 0.02	7.06 ± 0.08	6.82 ± 0.18	7.19 ± 0.14
Alb	$g.dL^{-1}$	3.03 ± 0.06	3.14 ± 0.07	3.12 ± 0.13	3.15 ± 0.12
Glob	$g.dL^{-1}$	3.70 ± 0.06	3.92 ± 0.04	3.70 ± 0.18	4.05 ± 0.16
BUN	$mg.dL^{-1}$	16.29 ± 0.03	18.85 ± 0.09	16.65 ± 0.37	19.04 ± 0.36
Chol	$mg.dL^{-1}$	53.81 ± 0.02	53.87 ± 0.05	53.63 ± 0.18	53.59 ± 0.20
Trigly	$mg.dL^{-1}$	26.46 ± 0.12	37.46 ± 1.53	27.74 ± 0.61	39.30 ± 0.12
AlAL	$u.L^{-1}$	28.72 ± 0.01	29.12 ± 0.19	27.98 ± 0.18	27.89 ± 0.52
AsAT	$u.L^{-1}$	72.97 ± 0.10	73.10 ± 0.18	73.22 ± 0.60	72.75 ± 0.58
TP-total protein;Alb-albumin;Glob-globulin;BUN-urea;Chol-cholesterol;Trigly-triglycerides;AsAT-Aspartate aminotransferase;AlAT-Alanine aminotransferase					

Obtained average values of the blood total protein (TP) ranged 6.73 to 7.19 $g.dL^{-1}$ (6.8 %). Albumin varying 3.03 to 3.15 $g.dL^{-1}$ (4.0 %), and globulin – 3.70 to 4.05 $g.dL^{-1}$ (9.4 %). The ratio albumin / globulin fractions of blood, known as "protein factor" (Fig. 1 and Fig. 2) did not show significant differences ($p>0.05$) between groups and diets.

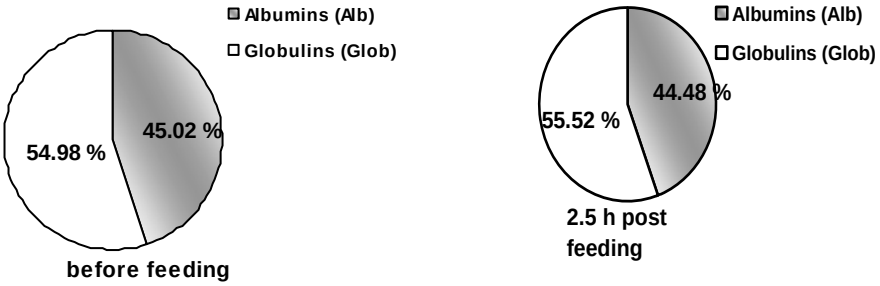

Figure 1. Protein ratio (%) for control group, fed SFM as protein source

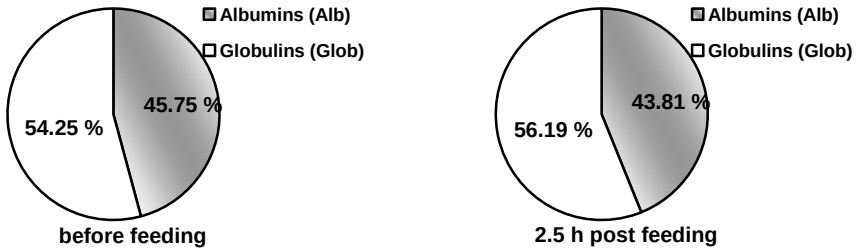

Figure 2. Protein ratio (%) for experimental group, fed DDGSc as protein source

No differences were observed among both diets in the BUN concentrations (table 4). The BUN values, although weren't statistically different among both diets, some authors reported that this metabolite were numerically higher for diets with higher DDGS inclusion rates compared to this with lower treatment. Although, the current values were within normal range for sheep and gives no indication of problems with using DDGS in the feeding program in regard of nitrogen utilization. The initial BUN concentrations (fig. 3) among the treatments (0.0 h) were very similar (16.29 vs. 16.65 $mg.dL^{-1}$) with 2.5 h after (18.85 vs. 19.04 $mg.dL^{-1}$) feeding (2.0 and 1.0 %). If compare the levels of BUN for experimental and control groups and the results be imposed on one axis, you can see how closely were the established factual values. This is in agreement with results from *Horn and Beeson (1969*), who observed no difference in BUN concentration in steers when supplemented basal diet with 5 % DDGS substituting cracked corn and urea. *Swenson (1977*) also did not detect any difference in BUN concentrations in goats diets supplemented with DDGS. Corresponding results was reported by *Gurung et al. (2009*) in goats (19.0-25.0 $mg.dL^{-1}$) for diets containing different levels DDGS. In contrast, heifers fed the 20 % DDG diets had higher BUN values than heifers receiving the diets with 10 % DDG (*Vander Pol et al., 2005*). In addition, they reported that heifers on all treatments had increased BUN values with time, as observed in the current trial. The BUN concentrations may be useful as an indicator of protein status within a group of animals and could help to find diets or identify problems with a feeding program (*Kohn et al., 2005*). Also, the BUN concentration has been shown to be related to energy intake and balance of protein and energy in diets (*Hagemeister et al., 1981*).

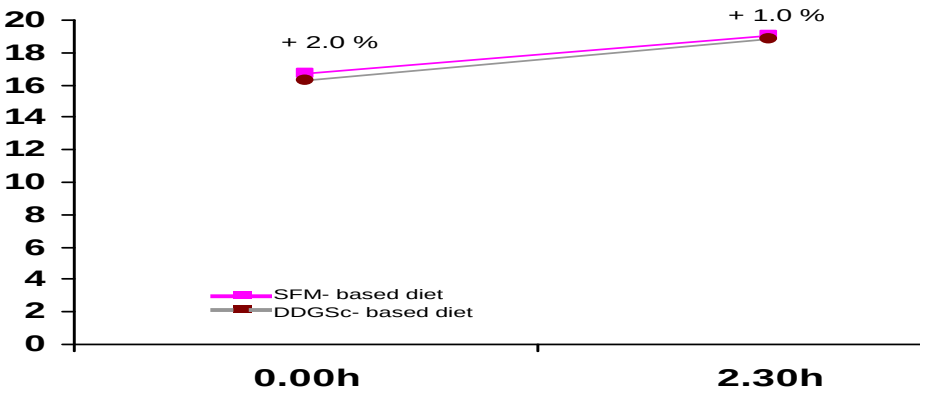

Figure 3. Dynamics of BUN concentration in the control and experimental group before and 2.50 h after feeding

The serum cholesterol concentration at the trial was no difference among both diets. Estimated values (53.81 vs. 53.63 mg.dL^{-1} before feeding and 53.87 vs. 53.59 mg.dL^{-1} 2.5 h post feeding) were within normal ranges for sheep (53-200 mg.dL^{-1}) (*Swenson, 1977*). Feeding fat to dairy cows almost always increases plasma cholesterol and the increase is independent of the degree of fatty acid saturation (*Grummer and Carroll, 1991*). The increased serum cholesterol levels in high DDGS-containing diets can be attributed to higher levels of fat in the diets due to DDGS inclusion. *Gurung et al. (2010)* in their study with goats demonstrated that serum cholesterol values increased linearly ($p<0.001$) as dietary amounts of DDGS increased in the diet, but stays within normal ranges.

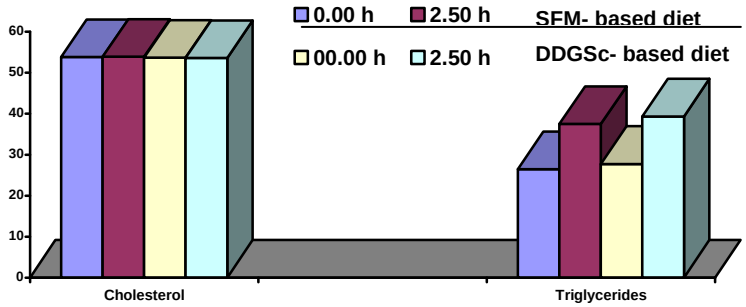

Figure 4. Dynamics of the concentrations of the lipid fractions in blood plasma in the control and experimental group

An absence of effect from DDGS inclusion on the other lipid fraction – triglyceride were indicative values before (26.46 vs. 27.74 mg.dL^{-1}) and 2.5 h after feeding (37.46 vs. 39.30 mg.dL^{-1}). In comparative terms, the values of triglycerides showed

(Fig. 4) that this indicator has significantly higher levels in the hours post feeding, as in the control group (37.46 vs. 26.46 mg.dL^{-1}) as in the experimental group (39.30 vs. 27.74 mg.dL^{-1}). The difference was near 29.4 %.
AlAT and AsAT are two of the most reliable markers of hepatocellular injury or necrosis. AlAT primarily exists in liver, but AsAT exists in various tissues like heart, liver, kidney and so on. So, liver's enzymes werealso not influenced significantly ($p>0.05$) and were at the normal levels in both treatments. During the trial, adding DDGSc tended to decrease AlAT, while did not affect AsAT significantly, which indicated that protein forage could protect the animal liver

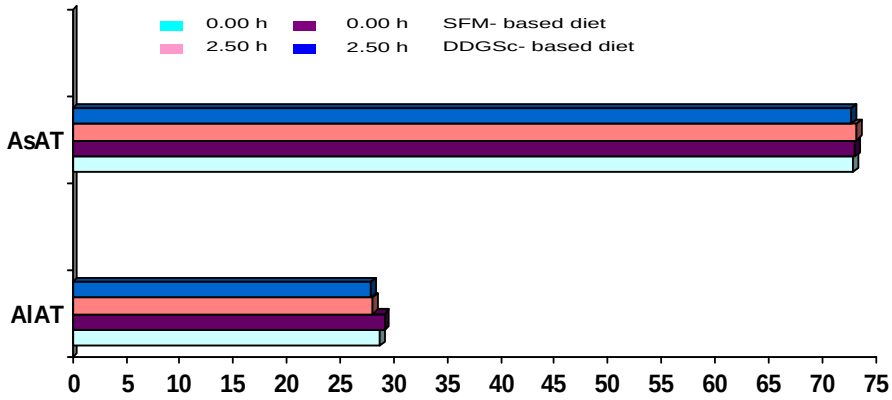

Figure 5. Dynamics of the enzymatic activity of aminotransferases AlAT and AsAT

under injury factors. The results obtained tends of reduction in both diets for the enzyme activities of AlAT (before feeding - 2.6 % and post feeding - 4.2%). The activity of other enzyme – AsAT, you can say that its varies within close range (73 u.L^{-1}) for both diets. Under the conditions of this experiment, the ratio between the activities of both enzymes (Fig. 5) values were in very close ranges (2.5 - 2.6) with no affect by the type of the protein source and diet.
The analyses of current trial shows that all controlled serum biochemical parameters allows to say that DDGSc does not cause significant negative changes and effects in the levels of total protein, albumin, globulin, urea concentration, cholesterol, triglycerides, as well as the activity of aminotransferases AlAT and AsAT. The obtained values of changes in the analyzed parameters can be applied to explain metabolism of fat in the body, liver function and the health status of animals in general, as well as utilization of diets and their nutrients, resp. animal performnce.

Conclusion

The obtained results showed that there were no any incidences of digestive disorders in both investigated groups. It's concluded that our choice of 38 % dried distiller's corn grains with solubles (DDGSc) as alternative nutritive component for small ruminants' rations and as a substitute of sunflower meal (SFM) had been suitable. On the base of these results we could go on to evaluate DDGSc effect on rumen degradability, digestion in the whole gastrointestinal tract, utilization of nutrients and animal production (meat, milk) in both quality and quantity.

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Uticaj korišćenja suve kukuruzne džibre (DDGSC) na neke biohemijske parametre kastriranih ovnova

M. R. Yossifov, L. K. Kozelov, M. A. Petkova

Rezime

U zadnje vreme, nove tehnologije za proizvodnju etanola dovele su do povećanja uloge ove industrije i proizvodnje sporednih proizvoda hrane (suva kukurzna džibra (DDG), glutena, itd). Nedostatak i kontinuirano povećanje cena tradicionalne hrane za životinje predstavljaju izazov za traženje njihove alternative. Istraživanja u ovoj oblasti su nedovoljno prisutna u Bugarskoj. Takođe je interesantno proučiti našu sopstvenu, bugarsku suvu kukuruznu džibru (DDGSc) kao komponente za ishranu malih preživara. Cilj našeg istraživanja bio je da se utvrde efekti DDGSc na kastrirane ovnove, odnosno biohemijske promene krvi. Dva obroka (Kontrolni obrok, CR i eksperimentalni obroki, ER) su formirana. Oni su sadržali 63% gotove smeše i 37% kabaste hrane. Glavne komponente gotove smeše bile su kukuruz i tritikale. Eksperimentalni obrok sadržao je DDGSc kao zamenu za suncokretovu sačmu u kontrolnom obroku. Oba obroka su ekvivalentna u pogledu sadržaja proteina. Hemijski sastav obroka je određen konvencionalnom

Weende analizom. Sledeći biohemijski parametri krvi (pre hranjenja i 2,5 sata nakon hranjenja) su proučavani: ukupni protein, albumin, globulin, urea, holesterol, trigliceridi, i aktivnosti enzima AsAT i AlAT. Svi biohemijski parametri su procenjeni korišćenjem Pentra 400 automatskog biohemijskog analizatora. Dobijeni rezultati su pokazali da nije bilo pojave digestivnih poremećaja u obe ispitivane grupe. Zaključeno je da je naš izbor DDGSc kao alternativne nutritivne komponente obroka ishrane malih preživara i kao zamena za suncokretovu sačmu u obroku bio pogodan. Na osnovu ovih rezultata možemo da nastavimo sa procenom DDGSc odnosno uticaja na varenje, razgradnju u rumenu, varenje u celom digestivnom traktu, iskorišćavanje hranljivih materija, proizvodnju (meso i mleko), sa stanovišta kvaliteta i kvantiteta.

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HISTOLOGICAL CHARACTERISTICS OF THE DUBSKA PRAMENKA PITUITARY GLAND DURING THE LACTATION PHASE

A. Katica[1], N. Mlaćo[1], A. Hodžić[2], K. Podžo[3], A. Softić[4]

[1]Veterinary Faculty , University of Sarajevo, Department of Histology and Embryology, Zmaja od Bosne 90, 71000 Sarajevo, Bosnia and Herzegovina.
[2]Veterinary Faculty , University of Sarajevo, Department for Physiology, Zmaja od Bosne 90, 71000 Sarajevo, Bosnia and Herzegovina.
[3]Veterinary Faculty , University of Sarajevo, Department For Reproduction and Physiology, Zmaja od Bosne 90, 71000 Sarajevo, Bosnia and Herzegovina.
[4]Veterinary Faculty , University of Sarajevo, Department for Animal Husbandry, Zmaja od Bosne 90, 71000 Sarajevo, Bosnia and Herzegovina.
Corresponding author: amela10katica@yahoo.com
Original scientific paper

Abstract: The pituitary gland is an endocrine gland, located in the ventral area of the diencephalon. It is connected to a hypothalamus, hypothalamic infundibulum, hence it is connected to the brain both through the nervous and vascular system. It is built of two parts: glandular- ***Adenohypophysis*** and neural- Neurohypophysis. Mamotroph cells (Cellula mamotropica) or epsilon cells, the cells of the frontal-distal part of the pituitary, secrete hormone prolactin which stimulates the secretion of milk, thus the number of these cells increases during gravidity and after the birth - during the lactation time. In our studies of the Dubska pramenka pituitary gland, conducted in the area of Central Bosnia Canton, where the sheep is bred in classical extensive conditions, in hill and mountain areas, microscopically we differentiated acidophil, basophil-chromophil as well as chromophobe glandular cells in pars distalis of the pituitary, which are of polygonal appearance, with large ball-like nuclei, centrally or eccentrically located.

Key words: Histological characteristics, hypophysis, Dubska pramenka, lactation

Introduction

Acknowledging the significance of the endocrine system and the role in controlling bodily functions, i.e. their harmonious activity with the nervous, immunological and reproductive system, we decided to run histological comparison of the pituitary gland and the lactocytes of the milk gland under the

conditions of holding of Dubska pramenka in BiH. The goal and the desire of the study was to describe the fundamental microscopic structure of the pituitary - pars distalis of Dubska pramenka, glandular cells pars distalis, to compare their morpha with the lactocytes - glandular cells of the milk gland and to accentuate the correlations, especially prompted by the fact that morphology-histology has been getting more intensive and comprehensive in the studies, including physiology and endocrinology (*Molik at al. 2010*).

Material and Methods

The materials required were taken in the field, in Central Bosnia Canton (Dubska pramenka is an indigenous sheep, village Dub, the Travnik area), and the studies were done at the Faculty of Veterinary of the University of Sarajevo. The histological assay of the pituitary characteristics and milk gland of Dubska pramenka were conducted during the lactation period. The total number of animals involved is eight (8). The samples of the pituitary, especially of the milk gland, were taken from several places, both from the right and left udder, paying close attention not to crush the active part of the gland - parenchyma. The samples were stored in plastic cups with lids filled with 10% formalin, until the moment of moulding in paraffin blocks. The moulding in paraffin blocks was done in a way that the samples of the milk gland were placed in 70% alcohol for two days, then in 96% alcohol for one day, and in 100 % alcohol for one day. The samples were transferred from this procedure into a mixture of 100% alcohol and toluol for two hours and after that only in toluol for four hours. The samples prepared were placed in paraffin I for five hours and paraffin II for twelve hours, after which the moulding procedure in paraffin blocks was completed. The paraffin blocks with the moulded samples of the pituitary and milk gland were cut using microtome, several series cuts 0.5 to 1.5 microns. The cuts were placed on glass slides, stained with haematoxylin eosin and azan, covered with cover glass and glued with Canada balsam. Histological assays were done using light microscope, under magnification of 100, 200 and 400 times. The microscopic examination included the entire preparation of the pituitary and the milk gland in order to get a full picture of the organs examined over given periods of time. The results of the assay were presented using descriptive interpretation of the histological preparations, making sure that the comparative presentation of the histological preparations is representative of our studies.

Results and Discussion

The pituitary (*Gl. pituitaria*) takes a central place in endocrinological happenings in the organisms of mammals - a "conductor" of endocrinological happenings. Structurally and functionally, it comprises of two parts: adenopituitary and neuropituitary. The adenopituitary part comprises of three parts: pars distalis, pars tuberalis and pars intermedia. Our studies focused on a detailed examination of pars distalis cells - chromophil, glandular cells in correlation with the milk gland during the lactation phase in Dubska pramenka, i.e. the morpha of the lactocytes. By observing the histological preparations of the pituitary under the light microscope during the lactation phase, we noticed the following characteristics: presence of chromophobic cells (non-coloured), and presence of acidophil cells, cells with red cytoplasmic granules and visible basophil cells with violet cytoplasmic granules. The descriptive assay determined a larger number of chromophil-acidophil cells during the lactation phase, which were grouped in a form of organized cords. Nuclei are extremely large and visible, of ball-like appearance, and cytoplasm is extremely acidophilic. The cells also show the phenomenon of degranulation and vacuolary excretion as a morphological form of increased activity.

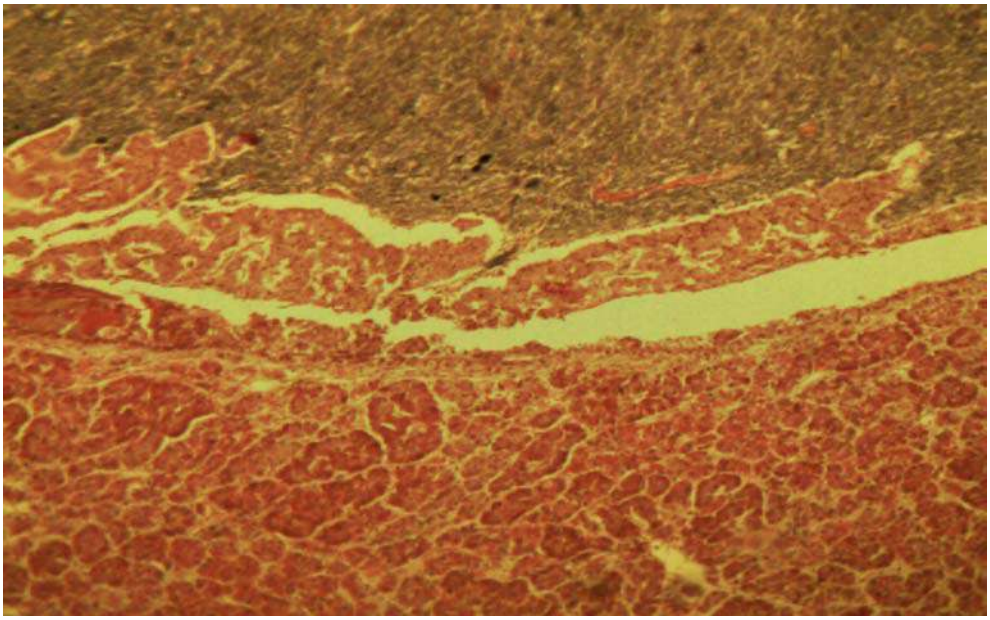

Figure 1. The pituitary gland of Dubska pramenka during the lactation phase, HxE 200x

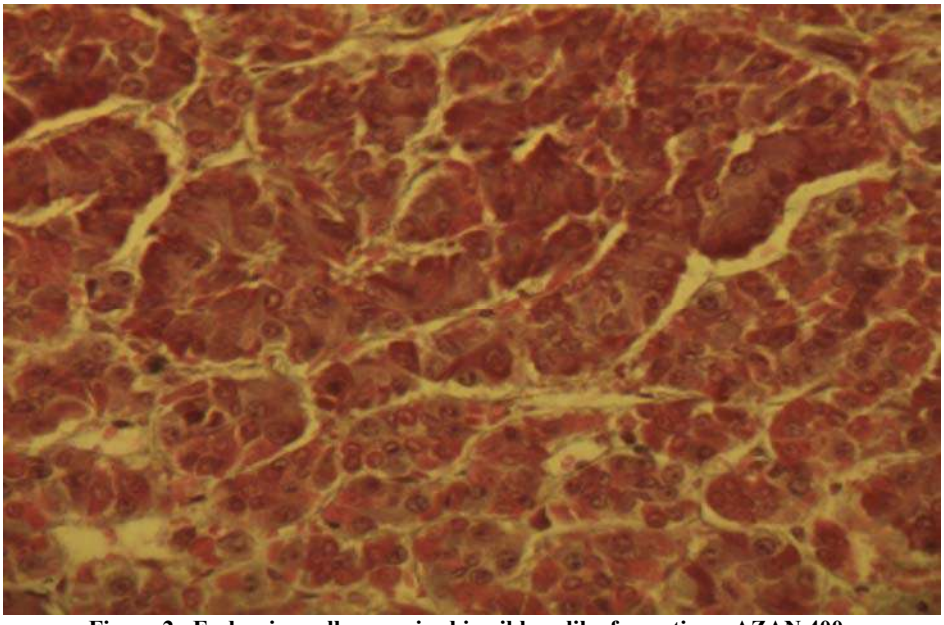

Figure 2. Endocrine cells organized in ribbon-like formations, AZAN 400x

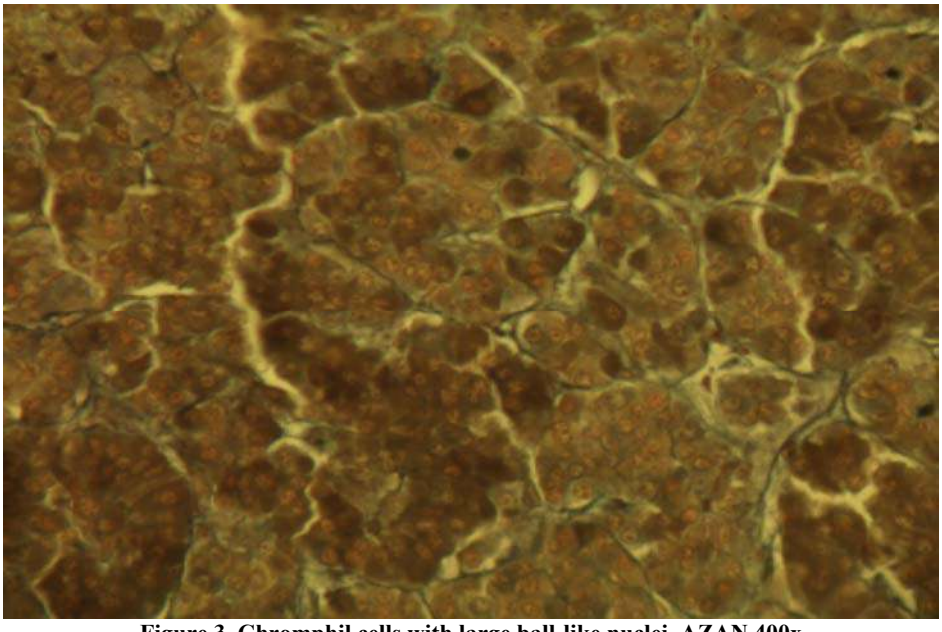

Figure 3. Chromphil cells with large ball-like nuclei, AZAN 400x

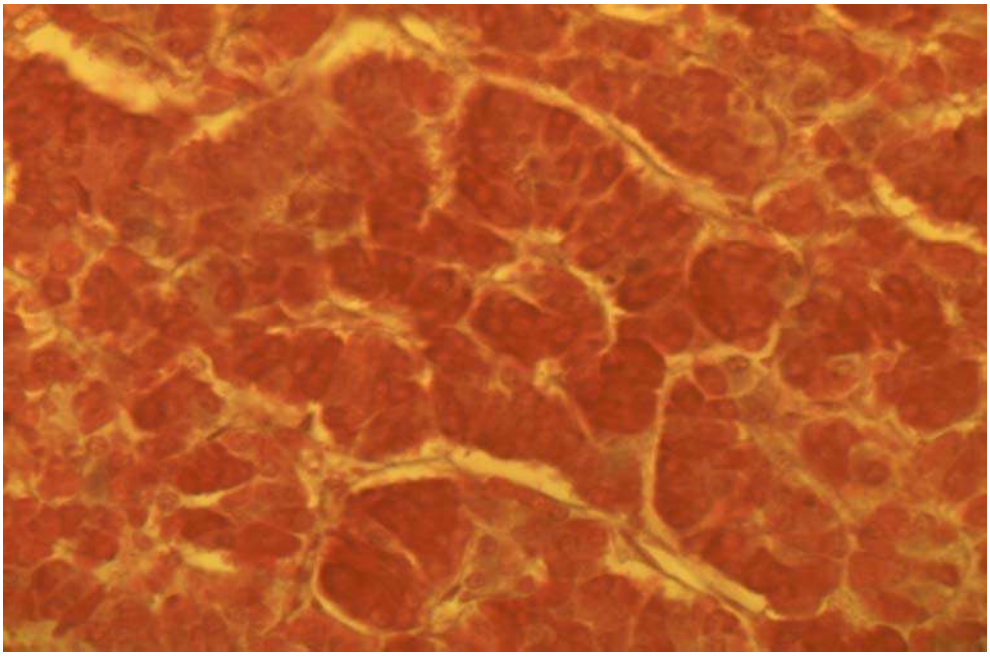

Figure 4. Acidophil cells in lactation phase with exceptionally acidophilic cytoplasm, HxE 400x

Mamotroph cells have the affinity for acidophil colours; they secrete the hormone "prolactine" (PRL), which induces milk secretion, *(Freeman et al. 2000)*, depending on the previous activity of estrogen, progesterone and placentary hormones. The interpretation of the histological preparation of the milk gland, using light microscope, large magnification, shows the parenchyma, which is made of numerous glandular acini, covered with prismatic lactocytes, whose height changes depending on the secretion phase.

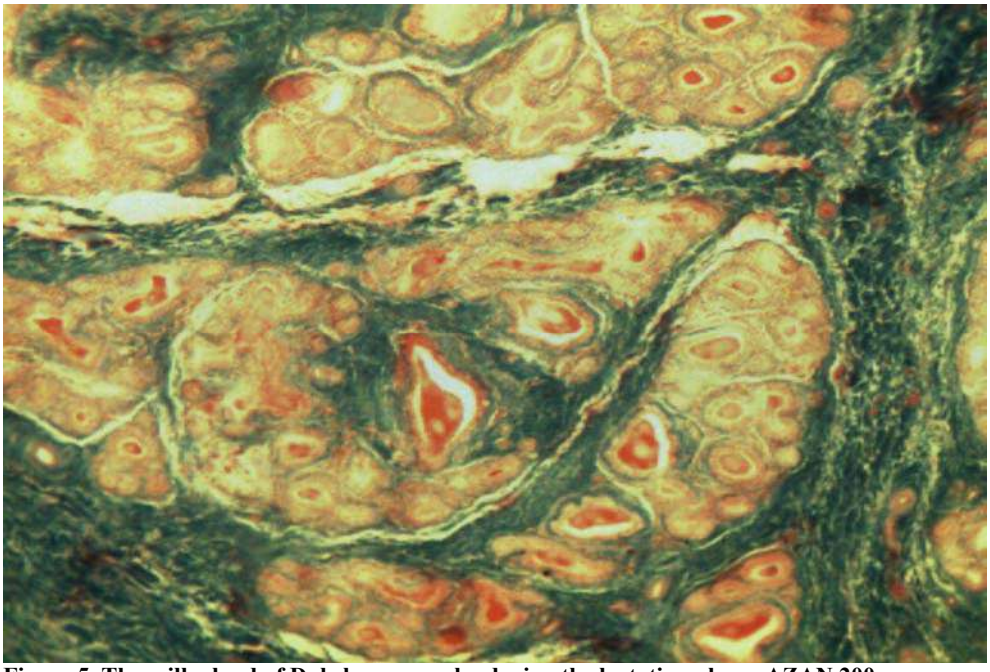

Figure 5. The milk gland of Dubska pramenka during the lactation phase, AZAN 200x

Smaller acini contain lactocytes in a form of high-prismatic cells, with darker cytoplasm and large ball-like nuclei. There is abundant infiltration of different cells between the acini. In some lobules, the glandular acini are of larger dimensions with high-prismatic lactocytes, lighter coloured cytoplasm and with smaller infiltration of cells in between. Then, there are lobules with large acini with lactocytes in the forms of low and flattened cells. There is a reduced number of cells in between the large follicles. The extracting ducts are wide, well developed, with or without contents, covered with high-prismatic epithelium. In the lumens of the follicles, there is a secretion of thinner or thicker consistency, with abundance of Corpora amilacea.

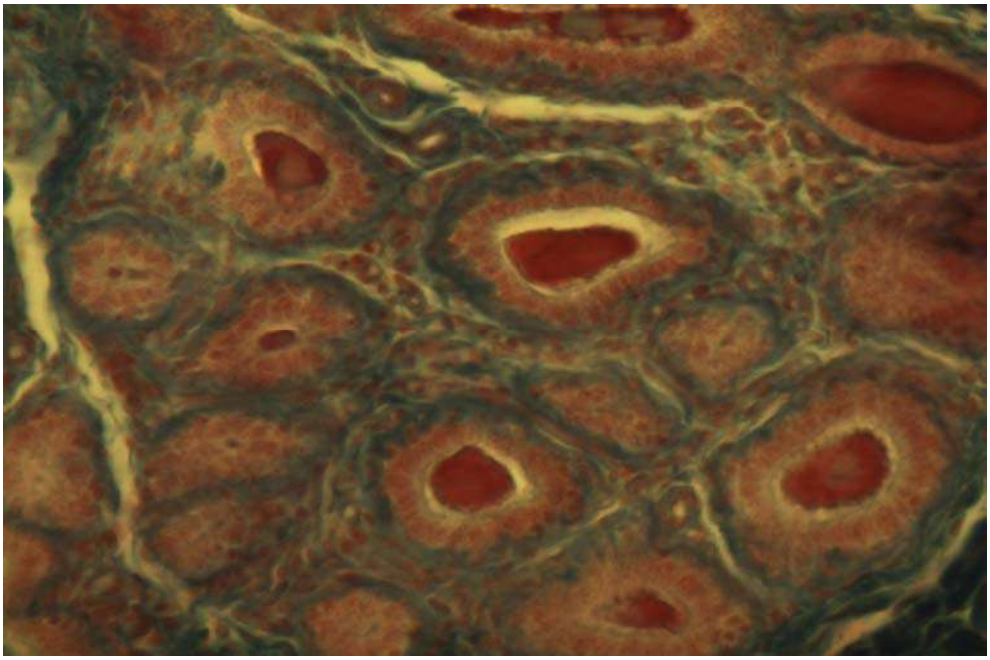

Figure 6. Lactocytes in the form of high prismatic cells and Corpora amilacea, AZAN 400x

The interlobular extracting ducts are well developed, wide and filled with contents and covered with a two-layer high-prismatic epithelium. The binding tissue is brought down to narrow septa between the lobules, so that the parenchyma of the gland is well developed. In the binding septa, there is a moderate amount of fat cells. Inside the lobules, there are glandular follicles of different sizes with excreted secretion.

Much of histological staining was used to differentiate cells that produce five (5) types of hormones, however, with little success. The methods of immunocytochemistry and electronic microscope are the only reliable techniques so far to differentiate these cell types (*Katica et al. 2010)*.

Our studies of the histological structure of the Dubska pramenka pituitary, in correlation with the milk gland during the lactation phase, aimed at description and interrelatedness of these organs. Based on the analysis of many factors, it is shown that changes in the level of light in the animals' environment have the most significant impact on the manifestation of sexual processes, i.e. on the function of neuroendocrine system (*Thiery et al., 2002*).

Our studies indicate that, during the lactation phase, there is an increased presence of chromophil - acidophil cells, grouped and easily observable by microscope in the form of cords. The epsilon cells are smaller, round, frequently mutually organized in a shape of a letter. Their cytoplasm grains are elliptic, 600-

900 nm in size and specifically stained with azocarmine They secrete the hormone prolactine or lactogenic hormone, which stimulates the milk gland to secrete milk, therefore the number of these cells in the adenopituitary increases during gravidity and after delivery. (*Kozarić, 1997; Junqueira et al., 2005; Eurell et al., 2006*) In order to detect cells of the distal part of the pituitary, it is very important to be familiar with functional modifications in addition to tinctorial characteristics. The presence of acidophil cells in pars distalis of the pituitary in Dubska pramenka, with our study, was brought into correlation with the histological structure of the milk gland during the lactation phase. Parenchyma of the milk gland is exceptionally developed, acini are filled with contents, that is, milk, and lactocytes are in a form of high-prismatic cells with large nuclei. The interstitium is less developed, built of connective tissue, in forms of cords, with presence of small number of adipocytes, which matches the findings of other authors (*Katica et al. 2012; Tatarczuch et al. 1996; Lee et al. 1998).*

Conclusion

Our research determined the following:

- Microscopic structure of pars distalis of the pituitary in Dubska pramenka during the lactation phase indicates exceptionally increased number of acidophil cells
- An appearance of nuclei of acidophil cells of the pituitary indicates their increased activity - hormone production
- During the study period, the acini of the milk gland are surrounded with lactocytes in a form of high-prismatic cells, filled with contents, while the interstitium is poorly developed, with less visible adipocytes
- The morpha of acidophil cells of the pituitary and the lactocytes of the milk gland during the lactation phase indicate mutual correlation and increased activity.

Histološke karakteristike hipofize dubske pramenke u fazi laktacije

A.Katica, N. Mlaćo, A.Hodžić, K. Podžo, A. Softić

Rezime

Hipofiza je endokrina žlezda, smeštena na ventralnoj površini diencefalona. Vezana je za hipotalamus, hipotalamusnom peteljkom - Infundibulum, tako da je nervno i vaskularno povezana sa mozgom. Sastoji se iz

dva dela, žlezdanog - Adenohipophysis i nervnog - Neurohipophysis. Mamotropne ćelije (Cellula mamotropica) ili epsilon ćelije, ćelije prednjeg - distalnog dela hipofize, luče hormon prolaktin, koji podstiče mlečnu žlezdu na lučenje mleka, te se broj ovih ćelija povećava u toku bremenitosti i posle porođaja - za vreme laktacije. U našim istraživanjima hipofize dubske pramenke, rađenim na području Centralne Bosne, gdje se ovca uzgaja u klasičnim ekstenzivnim uslovima držanja, u brdsko-planinskim delovima, u pars distalis hipofize mikroskopski smo diferencirali acidofilne, bazofilne - hromofilne, kao i hromofobne žlezdane ćelije koje su poligonalnog izgleda, krupnih loptastih jedara, centralno ili ekscentrično postavljenim.

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THE EFFECT OF PARITY ON PARTURITION PATTERN IN BULGARIAN WHITE DAIRY GOATS

Y. Aleksiev [1], S. Stoycheva [1], P. Zunev [1], Ts. Hristova [1], Ts. Maslev [1], G. Dimov [2]

[1] Institute of Mountain Stockbreeding and Agriculture, 5600 Troyan, V Levski str, Bulgaria
[2] Agrobioinstitute – 1164 Sofia, 8 Dragan Tsankov blvd.
Corresponding author: Yordan_Aleksiev@yahoo.com
Original scientific paper

Abstract: Diurnal distribution of kidding was studied in Bulgarian White dairy goats of different parity kidded in February. Does were divided according to their parity into three groups consisting of 56 primiparous goats, 85 second- and thrimiparous individuals and 91 multiparous animals, respectively. For all groups of does parturitions displayed a unimodal distribution with maximum kidding around midday and with the lowest percentages occurring over the dark hours. The majority of birth occurred between 06.00 and 20.00 h when kidded 90.92 % of the primiparous does, 95.53 % of secondiparous and trimiparouse goats and 92.11 % of fourth or greater parity individuals, respectively The peak of parturitions for all parity categories occurred between 14.00 and 16.00 h. During the night hours the incidence of deliveries were significantly lower ($P < 0.01$) compared to those recorded at day time. No differences ($P > 0.05$) were found in the hourly frequencies of kidding in regard to parity. It may be hypothesized that the diurnal rhythm of deliveries displayed by goats seems to be an evolutionary pre-set event favoring the survival of the newborn kids.

Keywords: goats, parity, birth distribution

Introduction

Animals, evolving in variable environment, have developed a range of strategies associated with adaptation to seasonal and diurnal environmental fluctuations. These adaptations include also seasonality in reproductive activity corresponded mainly with the changes in the photoperiod, diurnal modulations of environmental extremes, plants development and feed availability that all may be related to survivability of the species. In domestic animals parturition pattern may be further modified by the prevailing management practices. Observation on sheep (*Younis and El-Gaboory, 1978; Alexander et al, 1993; Aleksiev, 2007*) showed

that, regardless of breeds, parity of ewes and seasons of lambing, parturitions are uniformly distributed over the 24-h period. Conversely, in different goat breeds *(Bosc et al 1988; Awotwi et al., 1999; Ramirez et al., 1995; Romano and Piaggio, 1999; Aleksiev., 2008, 2011)* hourly distribution of kidding showed unimodal distribution and this parturition pattern was not influenced by the season of delivery, sex of the newborn, litter size and geographic locations. In all studies incidence of parturitions usually peaked around midday and most of deliveries occurred during daylight hours. Less or no kidding was reported in different goat breed during the nighttime hours.

Birth is the critical stage of reproductive cycle. From the practical point of view the prediction of diurnal rhythm of deliveries may help to optimize farm routine and to increase productive efficiency by reducing mortality rate in multiple births particularly in primiparous does having no maternal experience.

The aim of the current study was to examine diurnal distribution of kidding in relation to the parous category in Bulgarian White dairy goats

Material and methods

Does were reared on pasture during the grazing period (April – October) and kept in barn during the rest months of the year. During the confinement period chopped hay was administered twice daily at 0800 hour and at 1800 hour in the quantity ensuring, in fact, *ad libitum* access to roughages. Daily ration included 500 g/head balanced concentrate. Water and salt were freely available. Kidding took place in January – March when does were observed continuously over the 24-h. In order to minimize a possible effect of the photoperiod and/or other environmental variables on the time of parturition only data recorded in February were taken into account. Electric light in the barn was turned on in the late afternoon and switched off in early morning. Data from 232 natural and spontaneous kidding, collected in three consecutive years in Bulgarian White goats (similar genotype), were combined in order to investigate the parturition timing pattern. The year effect was not taken into account. Does were divided, according to their parity, into three groups consisting of 56 primiparous goats, 85 second- and thrimiparous individuals and 91 animals of fourth and greater parity.

Data regarding temporal dynamic of kidding were analyzed according to *Snedecor and Cochren (1989)*. Differences were assessed by a Student's *t* test for significance.

Results and Discussion

For all groups of does parturitions displayed a unimodal distribution with maximum kidding around midday and with the lowest percentages occurring over the night time. The majority of birth occurred between 0600 and 2000 h when kidded 90.92 % of the primiparous does, 95.53 % % of secondiparous and thrimiparouse goats and 92.11 % of fourth or greater parity individuals, respectively (Fig. 1). The peak of parturitions for all parity categories occurred between 1400 and 1600 h when kidded 20.45 % of primiparous, 21.52 % of secondi- and thrimiparous and 25 % of fourth and greater parity does. During the dark hours between 2000 and 0600 h the incidence of deliveries were significantly less ($P < 0.01$) compared to those recorded at day time. No births were recorded in primiparous does between 0000 and 0400 h. No differences ($P > 0.05$) were found in the hourly frequencies of kidding with regard to parity.

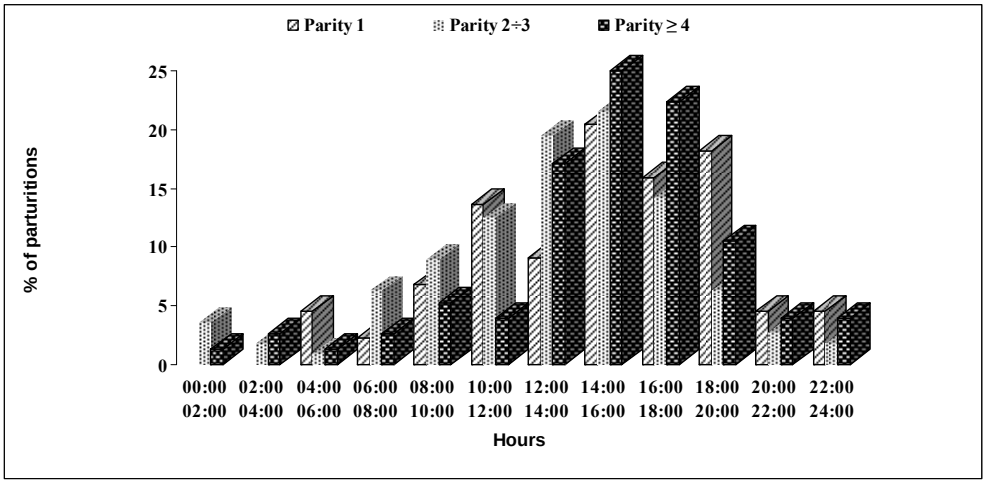

Figure 1. Hourly distribution of kidding in does of different parity.

Similar circadian distribution of parturitions was observed *by Awotwi et al (1999*) in West African Dwarf goats, *Konyali et al (2006)* in Turkish Saanen goats, *Yilmaz et al. (2012)* in local primiparous Dormuz goats, *Romano and Piaggio (1999)* in Nubian goats kidded in different seasons of the year. In all studies, regardless of the goat breeds and geographic location, hourly frequencies of kidding were concentrated during the day time hours with a peak of deliveries before noon. This parturition timing pattern differs from that found in the current and in our earlier studies (*Aleksiev, 2008, 2011*) in different goat breeds exhibiting the highest frequencies of births in the early afternoon. This discrepancy relating to

the peak of kidding within the day may be due to kidding season and/or the electric lighting in the barn during the dark hours that may modify hormonal secretion rhythm and initiation of labour. The absence of parturitions during the night hours in primiparous does may possibly be due to the small size of the group than to the age of the goats. No effect of goat parity and the number of fetuses carried on parturition timing pattern was found both in our and in all other cited studies.

Mellado et al. (2012) found some differences in parturition patterns in Murciano-Granadina goats kept under rangeland or pen conditions related mainly to the peak of kidding over the daytime In both rearing systems the majority of births occurred over the day time, achieving the peak frequencies between 0800 and 1100 h in intensive conditions and between 1100 and 1400 h in range conditions. These results showed that rearing system is able to affect only the peak of the incidence of deliveries within the day time without modifications in circadian parturition pattern. No births were recorded between 2300 and 0500 hours that correspond closely to the findings of *Otal et al. (2010)* who reported no births between 2200 and 1000 hours in the same goat breed. Effect of daily farm routine including feeding time and diet composition on circadian rhythm of parturitions have been observed in beef cattle and dairy cows (*Aoki et al., 2006; Stevenson, 1989; Jaeger et al., 2008*). Morning fed near term cows showed uniformly distribution of calvings over 24-h period, while in afternoon fed animals the majority of births occurred during daylight hours. In sheep feeding schedule and/or surrounding activity may also affect birth distribution (*Cobb and Gonyou, 1982; Hudgens et al., 1986*). These findings revealed the possibility to modify the existing parturition pattern through the changes of daily farm activity. The observed parturition pattern displayed by different goat breeds and parous category suggests the relationship between births distribution, changes in dark-light cycle and hormonal secretion rhythm.

The initiation of labor is associated with the activation of hypothalamic-pituitary-adrenal axes of the fetus following maturation of the central nervous system with advancing of gestation. These events are similar in mammalian species that can not account for the specificity of parturition timing pattern recorded in different goat breeds kept in different rearing systems and geographic locations. It is very likely that concentration of kidding during the daylight hours corresponds to the dark-light cycle and diurnal oscillations of effective envirnmental parameters determining the cooling power of the environment and the rate of heat loss. *Muller and McCutcheon (1991)* reported that newborn kids exhibited significantly lower metabolic activity and smaller capacity for heat production compared with the newborn lambs. Therefore, the concentration of kidding during midday hours may be accounted for, at least partly, by the limited thermoregulatory capacity of the newborn kids. During the day air temperatures usually achieve maximum values

that results in decline of heat dissipation from the body surface. Kidding during the warm day time hours appear to serve kids survival. The initial mechanisms governing the timing of delivery seem to be encoded in the genome *(Jenkin and Young, 2004)* and activates after occurring of certain prerequisite developmental events. In the case of goats the occurrence of labour may be further associated with diurnal oscilation of dark-light cicle, fluctuations of environmental extremes and thermoregulatory ability of the newborn kids.

Conclusion

Data derived from this study revealed that does of different parous category display a unimodal distribution of deliveries with maximum kidding in early afternoon and with the lowest percentages occurring over the dark hours. The majority of births occurred between 0600 and 2000 h when kidded 90.92 % of the primiparous does, 95.53 % % of secondiparous and thrimiparouse goats and 92.11 % of fourth or greater parity individuals, respectively. The peak of parturitions in all parity categories occurred between 1400 and 1600 h. During the night hours the incidence of deliveries were significantly lower ($P < 0.01$) compared to those recorded at day time. No differences ($P > 0.05$) were found in the timing of kidding in regard to parity.

It may be hypothesized that the circadian parturition pattern displayed by goats seems to be an evolutionary pre-set event favoring the survival of the newborn kids.

Uticaj pariteta na porođajne obrasce kod bugarskih belih muznih koza

Y. Aleksiev, S. Stoycheva, P. Zunev, Ts. Hristova, Ts. Maslev, G. Dimov

Rezime

Raspodela jarenja je ispitivana u populaciji bugarskih belih mlečnih koza različitih pariteta koje su se ojarile u februaru. Koze su bile podeljene prema paritetu u tri grupe koje su se sastojale od 56 koza u prvom partusu, 85 koza u drugom i trećem partusu i 91 koza koje su se jarile vise puta, respektivno. Za sve grupe koza, partusi su pokazali jednomodalnu raspodelu sa maksimalnim brojem jarenja oko podneva i sa najmanjim procentima tokom mračnih sati. Najviše partusa se dogodilo između 06.00 i 20.00 h kada se ojarilo 90,92% koza u prvom partusu, 95,53% koza u drugom i trećem partusu i 92,11% koza koje su se jarile

vise puta, respektivno. Vrhunac porođaja za sve kategorije pariteta dogodio se između 14.00 i 16.00 h. U noćnim satima učestalost porođaja je značajno niža (P <0,01) u odnosu na one koji su zabeleženi tokom dana. Nije utvrđena razlika (P > 0,05) u časovnoj frekvenciji jarenja u pogledu pariteta. Može se izneti hipoteza da ritam dnevnih porođaja kod prikazanih koza se čini kao evolucioni unapred određen događaj kojim se favorizuje opstanak novorođene jaradi.

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VARIABILITY OF PHYSICAL AND CHEMICAL PARAMETERS IN RAW SHEEP MILK DURING SEASONAL PURCHASE

N. Memiši[1], S. V. Moračanin[2], M. Žujović[3], N. Stanišić[3], Z. Tomić[3]

[1] AD Mlekara – Subotica, Tolminska 10, 24000 Subotica, Serbia
[2] Institut za higijenu i tehnologiju mesa, Beograd, Serbia
[3] Institut za stočarstvo, 11080 Beograd, Serbia
Corresponding author: memisin@mlekara.rs
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Abstract: This paper presents the results of investigation for the year and all seasons on the content of specific chemical parameters (milk fat, protein, lactose and dry matter without fat) and physical characteristics (pH, titratable acidity, specific gravity) in the aggregate samples of sheep milk during the two production years. The investigations were performed with collected sheep milk control samples that arrived at Subotica Dairy company from 10 collection sites which are located in the Banat region. Samples were taken daily during the period from mid-March to the end of September, and during the season of buying sheep milk. Chemical quality of milk is controlled on a daily basis at the laboratory for raw milk "Dairy" - Subotica on the apparatus CombiFoss 6200 FC. The total amount of milk that is arriving daily in the dairy during the full season of purchase was about 2500 kg, and slightly lower at the end of the season (August - September about 920 kg). Flocks of sheep, from which milk samples were obtained in the course of the entire production period were kept in similar (semi-extensive) conditions, while the grazing was the main meal during the summer and meadow and alfalfa hay with a small addition of ground corn, during winter. The average values of the analyzed physico-chemical parameters of raw sheep milk during the seasonal purchases were within the limits of standard quality for sheep milk. It is established significant influence of year and season of purchase on the content of some chemical parameters in sheep milk ($P < 0.01$)

Key words: sheep milk, milk fat, year, seasonal purchase

Introduction

Milk yield is polygenic trait caused by many genes that are directly or indirectly related to his exposure. Milk production is closely related with the

environmental factors, such as maternal nutrition before and after kidding, number of kids, climatic and soil conditions, care, rearing and many others. High hygienic quality of milk are required to protect the human health, maintenance of natural biological value of raw materials and providing proper technological process in the manufacture of milk.

In the past sheep farming and sheep milk production was far more massive and popular branch of livestock production than is the case today. It took place on a large pastures and meadows, hilly and mountainous areas, not least in the flat areas, as is the case in Vojvodina *(Maslovarić, 1987)*. However, in recent years, with economic progress, these areas, as well as sheep production is increasingly abandoned. Number of sheep rapidly decreases but the need and the demand for sheep milk and its products remain and even grow.

The quality of sheep milk in our conditions is much less studied in relation to the quality of cow's milk, because cow milk production significantly exceeds the rate of production of sheep milk and that most of the breeds of cattle is used for milk production. Another reason is the one that is in the world and in our country most breeds of sheep are bred for wool and meat production and they are not for milking, and the case is reversed for the cattle.

According to the FAO data published in 2009, the decrease of the world sheep flock in the recent decades (minus 10% from 1990 to 2007) is mainly due to Europe and Oceania who respectively lost 48% and 42% of their flocks between 1987 and 2007, when Asia and Africa respectively increased their flock of 50% and 53%. From the early 70's and in spite of the decrease of the sheep flock since 1990, the world sheep meat and milk productions both continued to increase significantly (about 60% of increase up to 2007). This has obviously been possible thanks to an increase of productivity for meat and milk sheep but also a significant conversion from wool to meat in the Southern hemisphere. Anyway, the sheep milk sector seems to be in a rather positive trend at world level.

Concerning the sheep milk production and according to FAO, Europe was still the leader region in the world in 2007 but, thanks to a higher growth during the previous ten years, the Middle East region may rapidly pass Europe (Table 1). Syria, the second country in the world after China had a 67% growth from 1997 to 2007.

Table 1. Recent changes for the main milk sheep regions in the world

	Production in 2007 (.000 t)	2007/1997 (%)
Main European countries	2599	7
Main Middle East countries[1]	2340	22
Main African countries	1304	17
China	107	0

Source: from FAO, 2009; (1) including Turkey

Northern America has no significant sheep milk production. Mexico, USA and Canada are estimated to have only less than 200 milk sheep farms with 25,000 ewes in total in 2010 *(Berger et al., 2010).*

The situation is similar in Oceania, but New Zealand seems to have a recent interest for the sheep milk production with a project of one farm with more than 10,000 ewes *(Berger, 2010).* Australia is mentioned to have some rare milk sheep producers mainly oriented towards yogurt making *(Idda et al., 2010).*

Sheep milk is less used for consumption but it is very suitable for the processing of various types of cheese (white, kachkaval, etc.) and fermented products. Sheep's milk is about 50% richer than cow's in dry matter. This happens primarily as a result of higher content of fat and protein, which makes it very suitable for the production of cheese because it provides almost twice the yield of the cow's milk. It is also very suitable for the production of sour milk products because it provides excellent consistency, which is very appealing to the consumers who are used to the smell and taste of sheep's milk and they gladly consume such products *(Djordjevic, 1987).*

Sheep lactation depends on the breed, diet, climate, and its duration is usually between 5-8 months. This means that the production of sheep milk in our conditions is highly seasonal, which really affects the capacity and capabilities of milk processing in the dairy industry. The problems associated with sheep farming as a whole also burden the production and processing of sheep milk, and the whole sheep production. These are before all extensive animal husbandry, low milk production, difficult working conditions, lack of manpower, which greatly reduce the number of sheep and the amount of sheep milk.

Material and methods

To determine the variation in quality of raw sheep's milk, during the seasonal purchases, collected milk that arrives to Subotica Dairy from ten purchase locations which are located in the Banat region has been tested. Samples were taken daily during the period from mid-March to the end of September, in other words during the season of purchase sheep's milk, since this is also the period when the sheep in our seasonal conditions are in the lactation period. The total amount of milk that is arriving daily in the season of purchase at its height was approximately 2600 kg, and slightly lower at the end of the season (August - September about 920 kg).

The physical properties tested were acidity (^{O}SH) and specific gravity. Specific gravity of milk is determined lactodensimetrom gauged at 20^{0} C.

Chemical composition of milk (milk fat, proteins, lactose and fat free dry matter) were controlled daily in the Laboratory for raw milk of the dairy plant AD "Mlekara" – Subotica using apparatus CombiFoss 6200 FC.

Flocks of sheep from which the milk was obtained were held during the whole production period in similar (semi extensive) conditions, where grazing was the main meal during the summer and meadow and alfalfa hay with a bit of ground corn, during the winter period.

The analysis of variance was used to establish the variability of investigated chemical parameters of milk caused by the impact of various factors. The following model was used to obtain the results of the variance analysis (least square means, determination of the model and significance of individual factors):

$$Y_{ikl} = \mu + G_i + M_k + F_l + eijkl$$

where:

Y_{ikl} = phenotypic value of certain traits included in the analysis,

μ – general mean value,

G_i – fixed effect of the year (i = 1, 2),

M_k – fixed effect of the month of the control (k = 1,..., 7),

e_{ikl} – other non-determined effects (random error).

All statistical analyses were done by applying appropriate procedures (Proc CORR, Proc GLM) within the statistical software package SAS. 9,1.

Results and discussion

Results of the investigation of the physic-chemical composition in collective milk samples, are presented per years of investigation and months of control (aggregate for both years) in Table 2.

Table 2. LSM for the basic quality parameters of raw milk per years and months of investigation

Year	N	Acidity (SH)	Protein (%)	Milk fat (%)	Lactose (%)	specific gravity	DMwF(%)
		LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE	LSM±SE
I	219	8.52±0.09[a]	5.44±0.03[a]	7.33±0.07[a]	4.55±0.03[NS]	10.39±0.08	10.52±0.09
II	233	8.67±0.10[b]	5.53±0.03[b]	7.45±0.08[b]	4.53±0.03[NS]	10.36±0.09	10.66±0.09
Month							
III	54	8.33±0.12	4.72±0.04	6.92±0.14	4.64±0.04	10.208±0.09	10.01±0.10
IV	60	8.42±0.13	5.09±0.05	7.14±0.13	4.54±0.04	10.298±0.08	10.28±0.09
V	62	8.24±0.13	5.39±0.05	7.11±0.10	4.53±0.04	10.373±0.10	10.57±0.08
VI	60	8.52±0.14	5.45±0.05	7.34±0.11	4.42±0.04	10.177±0.10	10.59±0.08
VII	62	8.79±0.12	5.42±0.04	7.42±0.09	4.45±0.03	10.411±0.12	10.57±0.08
VIII	62	9.03±0.14	5.76±0.05	7.69±0.09	4.50±0.04	10.263±0.09	10.91±0.08
IX	60	8.92±0.13	6.19±0.04	7.90±0.09	4.70±0.04	10.326±0.08	11.54±0.09
Statistical significance		*	***	***	NS	NS	***

[a,b] Values in the same column marked with different words are significantly different (***P<0,001; *P<0,05)
NS = not significantly; LSM = Mean corrected value; SE = The standard error; n = number of samples

The average value of milk acidity was 8.60 and ranged within 8.33 to 8.92 ^{0} SH.

The average content of milk fat during both study years was 7.37% and it gradually increases going from the beginning of the milk purchase (6.92%) until the end of the month of September (7.90%). The values obtained for dry matter without fat, protein and milk acidity throughout the season of purchase show certain variations, and it has been observed the values increase gradually at the end of season, while the values for lactose had the least variation and were not statistically significant (P <.05). In studies by other authors they have found similar trends of variation of various parameters in sheep milk during the lactation period. So *Antunac et al., (2011),* at the end of lactation period in sheep that are raised on island Pag found significantly higher milk fat content at the end of lactation period (8.2%) compared to the beginning of lactation (7.47%). Authors reported a significantly higher content of fat in sheep ranching in the Northeast compared to the southeast of the island of Pag. In the study of *Gonzalo et al., (1994),* milk fat content in milk of Churra sheep increased from forty-fifth (5.96%) to hundred-fiftieth day of lactation (8.20%). Milk fat content in milk of Chios sheep too, has been increased from seventh (5.76%) to twenty-sixth (7.76%) week of lactation *(Ploumi et al., 1998).*

A similar tendency of changes was found in the study conducted in sheep of Valle del Belice *(Cappio- Borlino et al., 1997)*. The protein content in milk Churra sheep increased from 45 (5.69%) up to 150 days of lactation (6.85%; *Gonzalo et al., 1994*). In the investigation of *Antunac et al., (2011)* who investigated the effect of paragenetic factors on the production and chemical composition of the milk of sheep which are raised on the island of Pag, it was found that the average proportion of protein in the milk of ewes significantly (P <0.001) increased during lactation, from 5.39% at baseline to 5.99% at the end. *Bencini and Pulina (1997)* conclude that the milk fat content in sheep milk significantly influenced by genotype and the lower (5.30%) in the milk of Romney sheep and significantly higher (9.10%) in the milk of sheep Comisana. *Ochoa-Cordero et al. (2002*) point out that the lowest content (5.1%), milk fat in Churra and the highest (12.6%) of Dorset sheep. The authors further state that a lower percentage of milk fat and protein in the milk of sheep selected for high milk production such as Awassi, East Friesian, Lacon et al.

Table 3. Results of analysis of variance of individual influence for the traits of the milk quality parameters

Factor	df	Protein (%)		Milk fat (%)		Lactose (%)		DMwF (%)	
		F	p	F	p	F	p	F	p
Intercept	1	104656	***	316182	***	2881901	***	314758	***
Year	1	56	***	99.2	***	5	0.02	41	***
Month	6	823	***	467.6	***	459	***	888	***

In the analysis of the effects of the study year and month of control, on changes in the chemical composition of milk in collective milk samples (Table 3), the precise determination of all variability sources which exhibit their influence within this effect, cannot always be made. No doubt, within the effect of the study year, very important are climatic factors and their direct impact on the quality of available food. It should also be mentioned that the effects of the year and month include series of effects which are associated with various zootechnical conditions present on the farm, and which may or may not vary from year to year. These influences can especially be exhibited on private farms, such was the case in this study, where there is often marked variability in regard to housing and nutrition conditions.

Conclusions

Based on the research on varying quality of raw sheep milk and its physico-chemical properties, during the season of purchase by AD Mlekara - Subotica, we can draw the following conclusions:

1. The average content of milk fat during both study years was 7.37% and it gradually increases going from the beginning of the milk purchase (6.92%) until the end of the month of September (7.90%). The values obtained for milk acidity ranged within 8.33 to 8.92 ^{0}SH.

2. The values obtained for dry matter without fat, protein and milk acidity throughout the season of purchase show certain variations, with an gradual increase by the end of season, while the value of lactose had the least variation and it was not statistically significant ($P < .05$). Analysis of variance confirmed that the variability of some chemical parameters in milk, was heavily influenced by the effects of individual factors analyzed (month of control and year when studies have been done).

3. Based on the obtained values of physical-chemical properties for raw sheep milk during the season of purchase, it can be concluded that all the parameters were within the limits of standard quality for sheep milk.

Aknowledgment

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Varijabilnost fizičko hemijskih parametara kvaliteta sirovog ovčijeg mleka u toku sezone otkupa

N. Memiši, S. Vesković-Moračanin, M. Žujović,N. Stanišić, Z. Tomić

Rezime

U ovom radu prikazani su rezultati ispitivanja uticaja godine i sezone na sadržaj pojedinih hemijskih parametara (mlečna mast, proteini, laktoza i suva materija bez masti), kao i fizičkih odlika (titraciona kiselost, specifična težina) u zbirnim uzorcima mleka ovaca u toku dve proizvodne godine.

Ispitivanja su izvršena kontrolom zbirnih uzoraka mleka od ovaca koje stiže u Subotičku mlekaru na 10 otkupnih mesta koja su locirana na području Banata. Uzorci su uzimani svakodnevno tokom perioda od sredine marta do kraja septembra meseca, odnosno tokom sezone otkupa ovčijeg mleka. Hemijski kvalitet mleka, kontrolisan je svakodnevno u laboratoriji za sirovo mleko AD "Mlekare" – Subotica na aparatu CombiFoss 6200 FC. Ukupna količina mleka koja je svakodnevno pristizala u mlekaru u punoj sezoni otkupa je bila oko 2500 kg, a nešto manja pri kraju sezone (avgust – septembar oko 920 kg). Stada ovaca od kojih je dobijano mleko su u toku celog proizvodnog perioda držane u sličnim (poluekstenzivnim) uslovima, pri čemu je paša bila osnovni obrok tokom letnjeg a livadsko i lucerkino seno uz mali dodatak prekrupe kukuruza, tokom zimskog perioda.

Prosečne vrednosti analiziranih fizičko-hemijskih parametara kvaliteta sirovog ovčijeg mleka tokom sezone otkupa bile su u granicama standardnog kvaliteta ovčijeg mleka. Utvrđen je značajan uticaj godine i sezone otkupa na sadržaj pojedinih hemijskih parametara ovčijeg mleka ($P<0,01$)

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APPLYING AN ECONOMETRIC MODEL TO FORECAST THE QUANTITY OF PRODUCED SHEEP MILK

T. Harizanova–Metodieva

Institute of Animal Science – Kostinbrod, Bulgaria, 2232
E-mail: ts_harizanova@abv.bg
Original scientific paper

Abstract: In 2011 were produced 89,296 tons of sheep milk in Bulgaria, there were 1,454.6 thousand sheep, of which 1,173.2 thousand sheep - mothers. The aim of this study was to forecast the quantity of sheep milk, produced in Bulgaria, using an econometric model. With the help of a regression model with a confidence level of 95% it was found that the predicted quantity of produced sheep milk for 2012 is 87,693 tons with the upper limit of variation of 91,893 tons and lower limit of 83,686 tons.

Key words: sheep milk, regression model, forecasting

Introduction

Sheep breeding is a traditional sub-sector in Bulgaria. It is a source of sheep milk, meat, leather, lanolin, wool. In our country in 2011 there were 1,454.6 thousand sheep, of which 1,173.2 thousand sheep - mothers. In that year 89,296 tons of sheep milk were produced. Sheep breeding in the country is characterized by extensive farming of animals, low average milk yield, small average number of animals in farms. Effectiveness of the sub-sector has been studied by a number of Bulgarian and foreign authors (*Guessous et al. 1989; Lupton, 2008; Wolfová et.al., 2011; Mihailova - Toneva, 2011; Mishev and Ivanova 2004*).

The aim of this study was to forecast the quantity of sheep milk, produced in Bulgaria, using an econometric model.

Material and Methods

A regression model was constructed with a confidence level of 95% using the Ordinary Least Squares Method (*Pindyck et. al 1991*). The regression model is as follows:

$y = c + b_1x_1 + b_2x_2^9 + a$

where

y - dependent variable

c – constant

x_1 and x_2 are the independent variables

b_1 and b_2 are the coefficients of the independent variables

a - error of the regression

By constructing an econometric model, it is possible on the basis of the time series to estimate a quantitative relationship between the various economic phenomena (*Ivanova, 2006*). Sources of information on which the econometric model is constructed are data from Ministry of agriculture and food, Republic of Bulgaria and National Statistical Institute, Republic of Bulgaria for the period 2001 - 2011 year. The evaluations of the parameters, testing of hypotheses and forecast have been performed by software Eviews 3.1 and Microfit 4.1.

The proposed econometric model forecasts the quantity of produced sheep milk in 2012 in Bulgaria. Independent variables in the model are indicators "Sheep milk produced in the previous year", "Dummy variable" and a constant. Dummy variable was introduced to exclude the years with abnormal changes in the quantity of produced sheep milk. These years are: 2004, 2005 and 2006. In the analyzed period the years in which the quantity of the produced sheep milk is greater than the quantity produced in 2002 are: 2004, 2005 and 2006. The reasons for this are mainly related to climatic conditions and better grazing. During these three years the reduction of sheep - mothers compared to 2002 is least (2.19% in 2004, 5.28% in 2005 and 6.33% in 2006). During the remaining years of the period there was decrease of the amount of the produced sheep milk compared to 2002.

The econometric model has the following mathematical expression:

LN_SH_MILK = C(1) + C(2).DUMMY + C(3).(LN_SH_MILK(-1)^9)

where the dependent variable is:

LN_SH_MILK - natural logarithm of the produced sheep milk /tons/;

The independent variables are:

DUMMY - dummy variable

LN_SH_MILK(-1) -the natural logarithm of the produced sheep milk /tons/ during the year t_{-1}

C(1) - constant of the econometric model

C(2), C(3) - coefficients of the independent variables.

Estimations of the parameters of the econometric model were found with the program Eviews 3.1. During the estimation of the parameters in the model, the null hypothesis is tested, according to which the regression coefficients corresponding to the variables are equal to zero. The standard error of regression (SE of regression), the coefficient of determination (R-squared), adjusted coefficient of determination (Adjusted R-squared) and others are calculated. Durbin-Watson coefficient is not taken into account due to the inclusion of lag variable (as an independent variable) of dependent variable in the model.

The next diagnostic tests to check the suitability of the econometric model, performed by software Microfit 4.1, are represented:

1. Serial Correlation. This is a test for serial autocorrelation in the residuals /Lagrange Multiplier Test/. In this test when the values of the probability of acceptance of the null hypothesis are greater than 0.05, the null hypothesis is accepted, which means that the autoregressive model of the residuals is statistically insignificant. The null hypothesis is that the coefficients in the autoregressive model of the residuals are zero (*Ivanova, 2006*).
2. Functional Form. The test is used to check the stability of the regression model and is based on Ramsey RESET Test. In Ramsey's RESET Test, when the values of probability of acceptance of the null hypothesis are higher than 0.05, the null hypothesis is accepted. The null hypothesis is that the coefficient of the squared theoretical value of the dependent variable in the auxiliary regression model is statistically equal to zero (*Ivanova, 2006*).
3. Normality. The test is used for accepting or rejecting the hypothesis of normal distribution of the residuals. The test is based on the coefficients of Skewness and Kurtosis. For values of probability greater than 0.05, the null hypothesis of that diagnostic test is accepted, which means that the residuals is normally distributed.
4. Heteroscedasticity. This is a test for autoregressive conditional heteroscedasticity in the residuals. In this test when the values of the probability of acceptance of the null hypothesis are greater than 0.05, the null hypothesis is accepted. The null hypothesis is that the residuals do not represent an autoregressive process with conditional heteroscedasticity (*Ivanova, 2006*).

Besides diagnostic tests of the econometric model, some detailed tests are conducted:

1. Test for serial autocorrelation in the regression of the residuals (Lagrange Multiplier Test) with the program Eviews 3.1 (5 lags are included).
2. Test for serial autocorrelation of the equation residuals up to specified number of lags /Correlogram of Residuals/. The test is computed with the program Eviews 3.1. In these two tests the null hypothesis is the same as the null hypothesis of the diagnostic test "Serial Correlation".
3. Autoregressive test for conditional heteroscedasticity in the residuals /Autoregressive Conditional Heteroscedasticity Test of Residuals (OLS Case)/. In this test when the values of the probability of acceptance of the null hypothesis are greater than 0.05, the null hypothesis is accepted. The null hypothesis is that there is no autoregressive conditional heteroscedasticity of residuals (*Ivanova, 2006*). The test was computed with the program Eviews 3.1.
4. White's Heteroskedasticity Test. This is a test for heteroskedasticity in the residuals from a least square regression (*White, 1980*). In this test when the values of the probability of acceptance of the null hypothesis are greater than 0.05, the null hypothesis is accepted. The null hypothesis is that there is no heteroskedasticity. The test was conducted with the program Eviews 3.1.
5. Test for stability of the model /CUSUM - Cumulative Sum of Recursive Residuals/. The test is conducted with the program Microfit 4.1.
6. Test for stability of the model /CUSUMSQ - Cumulative Sum of Squares of Recursive Residuals/. The test is conducted with the program Microfit 4.1.

The graphs of the recursive residuals of CUSUM and CUSUMSQ tests show if the chosen model is stable.

7. Error in the assessment of the model and the level of the standard error. This test is conducted to verify whether the graph of the error is between the two lines of the standard error. The test is conducted with the program Microfit 4.1.
8. Normal probability plot of residuals - due to the small number of observations a normal probability plot of residuals is represented. The plot is displayed with the program Eviews 3.1.
9. Evaluation of correlation coefficients between the variables in the model. For this purpose, a correlation matrix of the correlation coefficients between the included in the model independent variables is calculated. The values below +/-0.500 show absence of collinearity. The correlation coefficients between the independent variables in the model are calculated by the program Eviews 3.1.

A plot of the actual and fitted value of the dependent variable /Actual and fitted values/ is presented, which indicates the match between the actual and fitted values of the dependent variable. The graph is displayed with the program Microfit 4.1.

The natural logarithm of the estimated amount of the produced sheep milk in 2012 and the value of the standard error are displayed with the program Microfit 4.1. The exponent applied to the base of the natural logarithm, shows the estimated amount of sheep milk produced in 2012 and the upper and lower limits of variation. The upper and lower limits of variation have been found according to this formula: Prediction+/-1.96*standard error.

Results and Discussion

The probability of acceptance of the null hypothesis (Prob.) in table 1, is less than 0.05, consequently the null hypothesis is rejected and therefore the regression coefficients in front of the variables in the model are different from zero. The coefficient of determination (R-squared) and Adjusted R-squared are high in value.

Table 1. Estimation of the parameters of the econometric model

Dependent Variable: LN_SH_MILK				
Method: Least Squares				
Sample: 2002 2011				
Variable	Coefficient	Std. Error	t-Statistic	Prob.
C	11.71592	0.073450	159.5083	0.0000
DUMMY	0.267559	0.018413	14.53074	0.0000
LN_SH_MILK(-1)^9	-1.03E-10	2.27E-11	-4.521606	0.0027
R-squared	0.970239	Mean dependent var		11.45256
Adjusted R-squared	0.961735	S.D. dependent var		0.114031
S.E. of regression	0.022306	Akaike info criterion		-4.524599
Sum squared resid	0.003483	Schwarz criterion		-4.433823
Log likelihood	25.62299	F-statistic		114.1022
Durbin-Watson stat	1.823883	Prob(F-statistic)		0.000005

By replacing the constant and the coefficients with their theoretical values it is obtained the following econometric model (Table 1):
LN_SH_MILK = 11.71592 + 0.2675591*DUMMY - 1.03e-10*(LN_SH_MILK(-1)^9)

The diagnostic tests of the econometric model are presented in table 2.

Table 2. Diagnostic tests of the econometric model

Diagnostic Tests		
* Test Statistics * LM Version * F Version *		
* A:Serial Correlation*CHSQ(1)= .094651[.758]*F(1, 6)= .057333[.819]*		
* B:Functional Form *CHSQ(1)= 3.2745[.070]*F(1, 6)= 2.9213[.138]*		
* C:Normality *CHSQ(2)= .36288[.834]* Not applicable*		
* D:Heteroscedasticity*CHSQ(1)= .018061[.893]*F(1, 8)= .014475[.907]*		

The probability of acceptance of the null hypothesis of the four diagnostic tests (Serial Correlation, Functional Form, Normality and Heteroscedasticity) in Table 2 are larger than 0.05.

The conducted detailed tests also showed that the selected model is stable and it can be used to forecast the amount of produces sheep milk in 2012:

- Probability values of tests for serial autocorrelation in the equation residuals /Lagrange Multiplier Test and Correlogram of Residuals/ for acceptance of the null hypothesis are greater than 0.05, therefore the null hypothesis is accepted, which means that the autoregressive model of residuals is statistically insignificant.
- Probability values of the test for Autoregressive Conditional Heteroscedasticity of Residuals (OLS Case) for acceptance of the null hypothesis are higher than 0.05, therefore the null hypothesis is accepted.
- Probability values of the White Heteroskedasticity Test for acceptance of the null hypothesis are greater than 0.05, therefore the null hypothesis is accepted.
- The graphs of the recursive residues CUSUM and CUSUMSQ (Figure 1 and Figure 2) show that the selected model is stable.

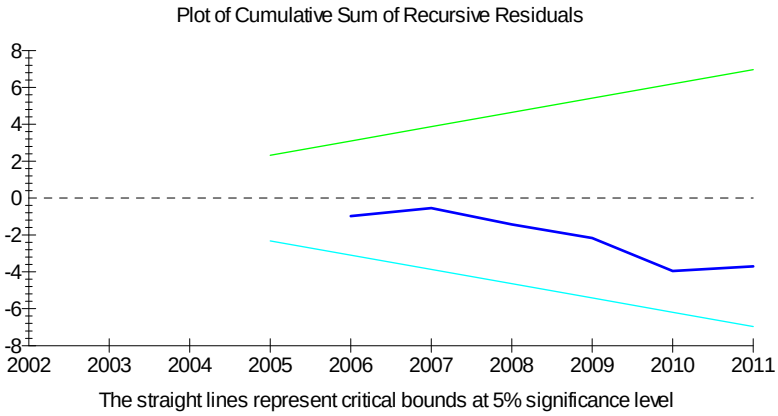

Figure 1. CUSUM - Cumulative Sum of Recursive Residuals

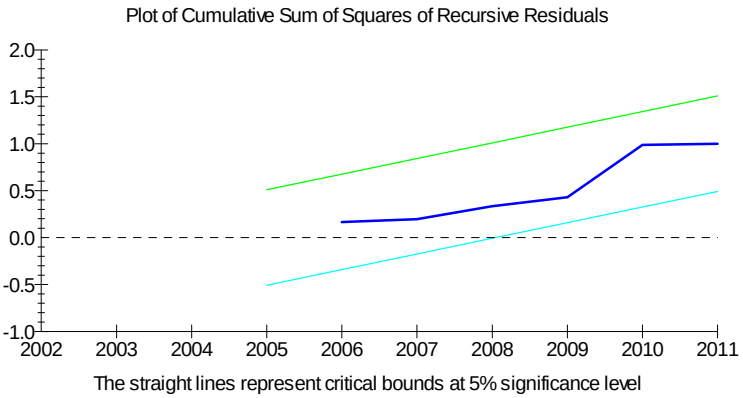

Figure 2. CUSUMSQ - Cumulative Sum of Squares of Recursive Residuals

- The plot of residuals falls between the two standard error bands.
- The normal probability plot of residuals shows a relatively normal distribution (Figure 3).

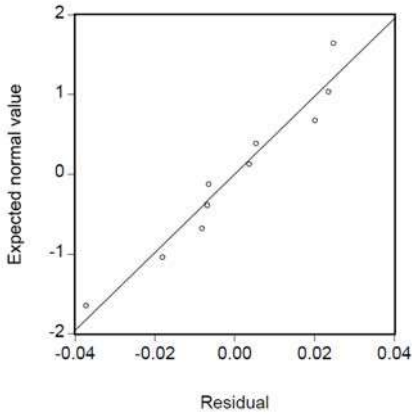

Figure 3. Normal probability plot of residuals

- The coefficients of correlation between the included independent variables in the model are below +/- 0.500, which means that the requirement for absence of collinearity is observed.

Figure 4 shows the Actual and fitted values of LN_SH_MILK.

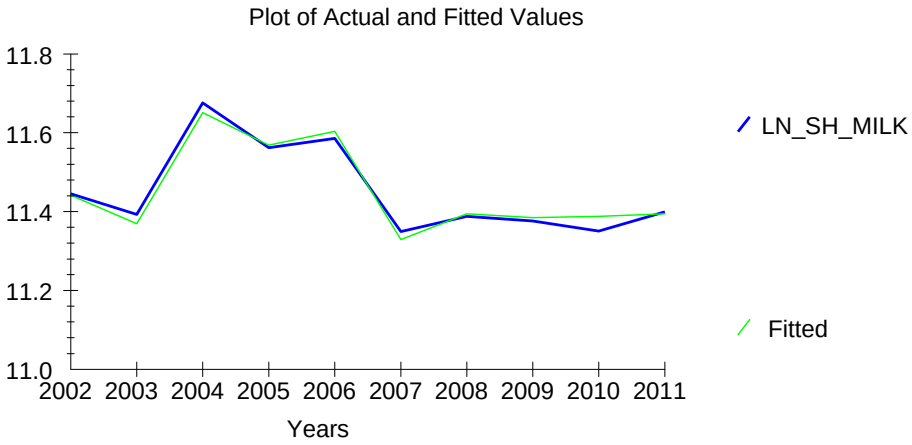

Figure 4. Actual and fitted values of LN_SH_MILK

It is found that the actual and fitted values of LN_SH_MILK have relatively good match.

The forecast of LN_SH_MILK for 2012 is based on a Single Equation Static Forecasts. It was found that the forecast for LN_SH_MILK for 2012 is 11.3816 and the standard error is 0.023866 (Table 3) and the estimated amount of the produced sheep milk in 2012 was 87,693 tons with an upper limit of variation of 91,893 tons and 83,686 tons lower. The exponent applied to the base of the natural logarithm in table 3, shows the estimated amount of sheep milk produced in 2012 and the upper and lower limits of variation. The upper and lower limits of variation have been found according to this formula: Prediction +/- 1.96*standard error.

Table 3. Single Equation Static Forecasts for LN_SH_MILK

Observation	Actual	Prediction	Error	S.D. of Error
2012	*NONE*	11.3816	*NONE*	.023866

Figure 5 shows the actual and Single Equation Static Forecast of LN_SH_MILK for 2012.

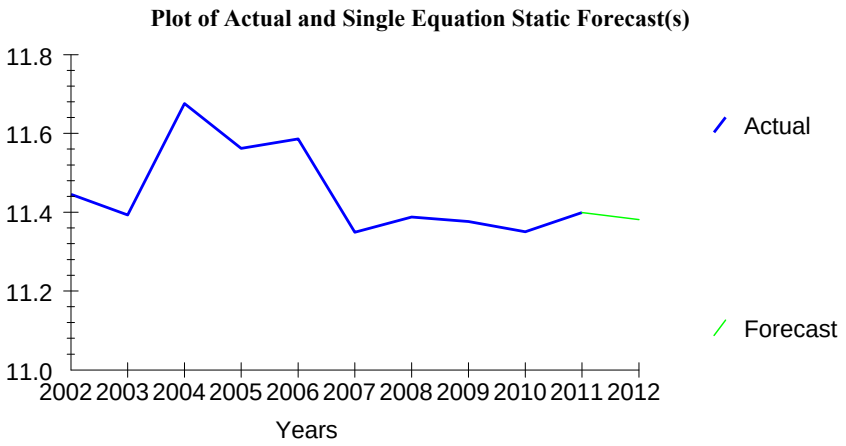

Figure 5. Plot of Actual and Single Equation Static Forecast of LN_SH_MILK

Conclusion

- The results of the diagnostic and detailed tests of the proposed econometric model indicate that the model is suitable for forecasting the quantity of produced sheep milk.
- The forecasted quantity of sheep milk produced in 2012 is 87,693 tons with an upper limit of variation of 91,893 tons and 83,686 tons lower.

Acknowledgement

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Primena ekonometrijskog modela za predviđanje količine proizvedenog ovčijeg mleka

T. Harizanova–Metodieva

Rezime

U 2011 je proizvedeno 89.296 tona ovčijeg mleka u Bugarskoj. Po podacima je bilo 1.454,6 hiljada ovaca, od kojih 1.173,2 hiljada ovaca - majke. Cilj ove studije bio je da se predvidi količina ovčijeg mleka, koji se proizvodi u

Bugarskoj, koristeći ekonometrijski model. Uz pomoć regresije sa nivoom verodostojnosti od 95%, utvrđeno je da je predviđena količina proizvedenog ovčijeg mleka za 2012 iznosi 87.693 tona, sa gornjom granicom varijacije od 91.893 tona i donjom granicom od 83.686 tona.

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CARCASS PARAMETERS AND YIELD OF SAANEN MALE KIDS

V. Simin[1], I. Pihler[1], M. Krajinović[1], M. Žujović[2], V. Tomović[3]

[1] Faculty of Agriculture, University of Novi Sad, Department of Animal Science, Square of D. Obradovića 8, 21000, Novi Sad, Republic of Serbia
[2] Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080, Belgrade-Zemun, Republic of Serbia
[3] Faculty of Technology, University of Novi Sad, Bulevar cara Lazara 1, 21000 Novi Sad, Serbia
Corresponding author: vladislav.simin@stocarstvo.edu.rs
Original scientific paper

Abstract: Saanen goat is breed for milk production but some number of male and female kids goes on slaughter. The aim of this study was to established carcass parameters and carcass yield of Saanen male kids. Ten Saanen male kids were obtained from one farm. Kids were feed *ad libitum* with milk replacer instead of goats milk, concentrate and alfalfa hay also *ad libitum*. On day 74, feed was withdrawn overnight and the kids were transported to the Institute of Animal Husbandry in Zemun where slaughter and measurements were taken 75th day. Statistical analysis i.e. descriptive statistic (average mean, standard error, standard deviation, coefficient of variation, minimum and maximum) was conducted using the software Statistica 12. Before slaughter is measured live weight (average 20,35kg) and after slaughter warm carcass without giblets (average 10,56kg), mass of organs (liver 0,46kg; lungs 0,45kg ; heart 0,10kg; spleen 0,04kg; omentum 0,40kg; testicles 0,06kg; waste 4,14kg; small intestine 0,57kg; legs 0,75kg; skin 1,84kg). The average carcass yield was 51,85%. Conclusion is although Saanen goats used for milk production, the results from this study show that it has good carcass parameters and yield and further research is needed. That is planned as a part of scientific project financed by national Ministry of Science.

Key words: Saanen kids, carcass parameters, yield.

Introduction

The world's goat population was around 720 million in 2000, with over 60% of that found in Asia and more than 95% in developing countries, with annual meat production of around 4.2 million metric tons *(FAO, 2003)*. Since 1985, there has been a significant increase (48%) in goat numbers all over the world *(FAO, 2001)*.

Naude´ and Hofmeyr (1981) described the goat population as comprised of four types, i.e., fiber goats (e.g., Angora and Cashmere), dairy goats (e.g., Saanen, Toggenburg and Nubian), meat goats (e.g., Boer) and feral goats.

Goats are raised in many countries all over the world due to numerous advantages that these animals provide, including: large capacity of acclimation, disease resistance, the ability of crosing long distances and useing a variety of foods, modest requirements in watering and feeding, and easily reproduce *(Krajinović, 2006)*.

Today in Serbia, the total number of goats are grown on private holdings, which usually run 5-10 animals, and whose products are used for subsistence production. It is estimated that only about 1% of farms breed more goats 50 – 200. For breed structure there is no accurate data, but *Žujović et al. (2005)* estimate that the situation as follows: 3% Saanen, Alpine, the average milk yield 480 l / lactation, 15% of domestic white goats, average milk yield 400 liters / lactation, 35% of goats from the Balkan region, the average milk yield 220 l / lactation, 35% of various crosses, the average milk yield 260 l / lactation, 12% Balkan goat from the higher areas, the average milk yield 120 l / lactation. In Serbia, the only support in goat production are farmers.

Saanen goat is the ideal model of dairy goat with high milk production and because of that last import into Serbia was 2009 of about 150 head. In good conditions and nutrition during one lactation period produce up to twenty times more milk than their body weight. Because of its high milk production, breeding goats is greatly appreciated and is now grown in many countries of the world. Successfully grown in intensive conditions, where produce large amounts of milk. Her fertility is also very high, more than 200%. The average milk production of goats older than 30 months of lactation of 280 days is about 800 kg. Selected herd of goats has milk production of 1000 kg or more. In some record-holder goats, milk yield is often greater than 5 kg per day.

In recent years Saanen breed is more and more popular and raised primarily for milk production, but as a result of this intensive production a large number of male and some female kids is used for slaughter. Male kids have more mass at birth and rapid growth before weaning about 10-15% more the female kids. Body weight and weight gain in kids directly affect the time of weaning and feeding regimes. Weaning is the stress and poor growth manifested, and sometimes come to a complete stagnation of weight gain and weight loss if it is carried out rapidly. What is the consumption of milk before weaning higher, the stagnation after weaning is pronounced. Stress due to weaning is lower in female than in male kids *(Mioč and Pavić, 2002)*.

Given the fact that in our country there are few works which explore themes of fattening and slaughter traits of kids, the aim of this study is to determine the characteristics of slaughter of Saanen breed.

Materials and Methods

For this study randomly were taken 10 male kids Saanen breed, with average age of 75 days from goat farm "Beocapra" from Kukujevci (Table 1). Goat production is carried out in the intensive system. The farm has more than 250 does and other categories. Kids are separated from the goats immediately after kidding and fed with colostrums in the first week and gradually turn the milk substitute that consume *ad libitum*. Between 20 and 25 days of life are given an alfalfa hay and concentrate *ad libitum*. The average consumption of milk replacer per kid is between 2.8 and 3 l. Kids are weaning after 2 months of age in groups. Daily gain of kids was about 212 grams per day (Table 1).

Table 1. Experimental design for the study with average mean and variability of weight at birth and at slaughter (kg) and average daily gain (g).

Age (days)	Type of birth	Birth weight (kg)	Weight at slaughter (kg)	Average daily gain (g)
83	T	4.9	19.8	179.52
79	T	5	24.6	248.10
79	T	4.9	18.15	167.72
78	T	4.2	22.9	239.74
78	Tr	3	17.3	183.33
76	T	4.2	23.2	250.00
75	T	4.8	21.8	226.67
74	S	5.7	20.5	200.00
70	T	3.3	21.7	262.86
66	T	3.9	14.6	162.12
$\overline{X}$		4.39	20.46	212.01
SE		0.26	0.97	11.92
SD		0.83	3.05	37.70
CV		18.92	14.94	17.78
Min		3.00	14.60	162.12
Max		5.70	24.60	262.86

S-single; T-twins; Tr- triplets; $\overline{X}$ -mean; SE-standard error; SD-standard deviation; CV-coefficient of variation; Min-minimum; Max-maximum.

Slaughter was carried out on 2nd June 2011 at the Institute of Animal Husbandry in Zemun in the slaughterhouse. Kids were weighed and then fasted for 18 h with free access to water and weighed again immediately prior to slaughter a digital scale with a precision of 5 grams. After the slaughter was measured hot carcass without giblets and a mass of warm carcass (before cooling) after classical treatment of slaughter, after the removal of offal. Classic slaughter processing involves evisceration without failure, cutting lower parts of the front and back legs below the hock, the separation between the cutting head of the first cervical vertebra and the occipital bone, and remove the skin. Then was measured weight of internal organs such as the liver, lungs, heart, spleen, tissues, white kidney, small intestine, waste. The waste is colon and rumen content. In addition to the internal organs, it measures the mass of the legs (with hooves) and leather. Mass of all these organs are measured individually with digital scale with a precision of 2 grams. After that, carcasses were chilled at 4°C for 24 h.

To calculate the dressing percentage is used a mathematical formula that represents the ratio of hot carcass weight multiplied by 100 and live weight before slaughter.

Contributions of organs in the carcass was obtained by applying a mathematical formula that represents the ratio of mass of organs and live body weight, multiplied by the number 100 that was expressed in units (%).

Using the computer program Statistica 12 parameters were calculated descriptive statistics such as mean value, standard error, standard deviation, coefficient of variation, and minimum-maximum. Effect of fixed factor (age at slaughter) on the weight at slaughter was established by application of ANOVA analysis (single dimensional classification).

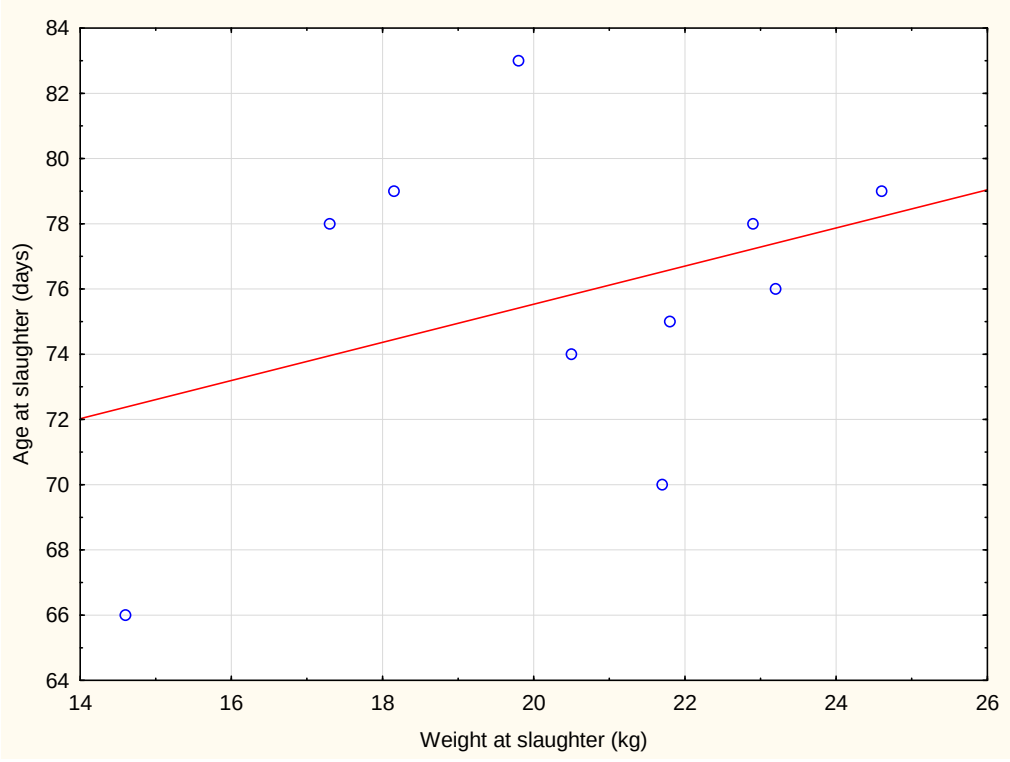
Results and Discussion

Average birth weight was 4.39 kg and average weight at slaughter 20.46 kg (Table 1). The birth weight of a kid depends primarily on the conformation and size of the adults of the breed to which it belongs *(Morand-Fehr, 1981)*. One of the major influences on growth of goats is the mature size of the sire and dam; generally, the progeny of large breeds grow faster than the progeny of small breeds *(McGregor, 1985)*. A significant effect of breed type on daily live weight gain has also been reported by *Dhanda et al. (1999a)*.

Difference between ages at slaughter wasn't affected on weight at slaughter. With an increase in age/body weight, a significant ($P < 0.05$) increase was

observed in carcass characteristics, i.e. dressing percentage, carcass length, eye muscle area and subcutaneous fat thickness reported *Dhanda et al. (2003)*. Similar results have also been reported by *Dhanda et al. (1999a)*. The castration of the Chevon kids was one of the contributing factors (others being age and carcass weight) for the higher subcutaneous fat deposition in Chevon compared to Capretto carcasses. The general concept that castrates are generally fatter than entire males is also applicable to goats *(Hogg et al., 1992)*.

Older kids were heavier before slaughter which attends on carcass characteristics (Graph 1.) Carcass characteristics and meat quality of goats can be affected by genotype *(Dhanda et al., 1999a,b; Oman et al., 2000)*, sex *(Hogg et al., 1992; Todaro et al., 2004)*, diet and production system *(Johnson and McGowan, 1998; Marinova et al., 2001)* and weight at slaughter *(Marichal et al., 2003)*. Nevertheless other factors could influence meat quality such as pre-slaughter treatment, carcass chilling, muscle type or ageing time *(Kadim et al., 2003; Webb et al., 2005)*.



Graph 1. Relationship between age and weight at slaughter

An average growth rate of 212.01 g per day (Table 4) achieved for the Saanen male kids in the present study was greater than 171 g per day in kids at average age 93 days at slaughter *(Dhanda et al., 2003)* and for Alpine and Nubian kids 110-140 g per day in a study by *Potchoiba et al. (1990)*. This difference could be due to the different breed types used in that study. Due to their greater average daily gains Boer x Saanen and Saanen x Feral kids reached the required liveweight for slaughter earlier than kids from other genotypes used in the study *Dhanda et al. (2003)*.

In presented study the dressing percentage was 51.85 % (Table 4). Similar values of the dressing percentage (based on EBW) of kids ranged from 51 to 54% reported *Dhanda et al. (2003)*. *Mahgoub and Lodge (1996)* reported similar values for the dressing percentage in Alpine kids and *Pihler et al (2013)* in Balkan kids 51,72% in same age like kids from this study. In some of the previous studies, the dressing percentage (based on the liveweight at slaughter) of goat kids had been reported to be around 46–48% in different goat breeds *(Colomer-Rocher et al., 1992; Hogg et al., 1992)*. *Van Niekerk and Casey (1988)* reported that significant differences between various goat genotypes for dressing percentage based on live weight were mainly attributed to the variations in the weight of stomach and intestinal contents at slaughter.

Table 3. Parameters of carcass characteristic in Saanen male kids

Parameters	N	$\overline{X}$	SE	SD	CV	Min	Max
Weight at slaughter (kg)	10	20.46	0.97	3.05	14.94	14.60	24.60
Hot carcass weight (kg)	10	10.56	0.51	1.63	15.41	7.97	12.99
Dressing percentage (%)	10	51.85	0.77	2.43	4.69	47.05	54.86
Cold carcass weight (kg)	10	10.33	0.51	1.60	15.48	7.77	12.68
Average daily gain (g)	10	212.01	11.92	37.70	17.78	162.12	262.86

Results for hot carcass weight and cold carcass weight were presented in Table 3. Similar results for hot carcass weight (11.2 kg) and cold carcass weight (10.9 kg) in different genotypes in male kids at age of 93 days reported *Dhanda et al. (2003)*. Loss in weight on chilling kids carcasses can be attributed to their thin subcutaneous fat cover (Table 3). Unless chilling rates are strictly controlled, goat carcasses due to their small subcutaneous fat cover, will always be at a risk of cold shortening (i.e. pre-rigor muscles rapidly chilled to temperatures below 10°C tend to contract extensively) *(Gonzalez et al., 1983)*.

Body components of empty body weight were presented in Table 4. The percentage contributions of various visceral organs, the liver and the skin, in this study, were similar to those reported for different breeds at similar liveweights

(Mahgoub and Lodge, 1996). *Dhanda et al. (2003)* reported that Boer x Angora kids had a significantly ($P < 0.05$) greater percentage of skin compared to other genotypes. This may be due to the fact that Angora goats have been reported to have greater fibre production than other breeds *(Riley et al., 1989)*. A significant ($P < 0.05$) decrease in the percentage of head and skin (based on EBW) with increase in age/body weight reported *Dhanda et al. (1999a)*. Significant effects of genotype and slaughter weight group on the proportions of visceral organs (liver, lungs, kidney and gastro-intestinal tract) were also reported by *Dhanda et al. (2003)*. *Gibb et al. (1993)* found a significant effect of breed type on the weights of visceral organs, as a proportion of liveweight. Saanen cross kids (Saanen x Angora and Saanen x Feral) and Feral kids deposited more internal fat than kids from other crosses *(Dhanda et al., 2003)*. Dairy breeds of goats tend to store more fat in visceral depots, rather than as carcass adipose tissue *(Gibb et al., 1993)*.

Table 4. Body components of kids (% of empty body weight-kg)

Organs	N	$\overline{X}$	SE	SD	CV	Min	Max
Liver	10	2.26	0.21	0.07	3.10	1.77	2.49
Lungs	10	2.20	0.32	0.10	4.55	1.82	2.68
Heart	10	0.50	0.05	0.02	4.00	0.42	0.59
Spleen	10	0.20	0.04	0.01	5.00	0.14	0.26
Omentum	10	1.90	0.65	0.20	10.53	1.11	3.30
Testicles	10	0.28	0.03	0.01	3.57	0.23	0.34
Waste	10	20.23	2.92	0.92	4.55	16.51	25.28
Small intestines	10	2.80	0.57	0.18	6.43	1.90	3.82
Legs	10	3.71	0.18	0.06	1.62	3.49	3.99
Skin	10	8.98	1.03	0.33	3.67	7.45	10.34

Conclusion

Although Saanen goat is breed used for milk production, the results from this study show that their kids has good carcass parameters and good dressing percentage where we compared with other breeds such as Alpine and Balkan goats. Weight at birth and weight at slaughter impact on yield and carcass parameters. Carcass characteristics such as cold and hot carcass weight are also good with comparison with other breeds that are raised in Serbia. So Saanen kids can be used for meat production.

Acknowledgment

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Klanični parametri i randman muške jaradi sanske rase

V. Simin, I. Pihler, M. Krajinović, M. Žujović, V. Tomović

Rezime

Sanska rasa je namenjena proizvodnji mleka ali određeni broj ženske i muške jaradi služi za proizvodnju mesa jer se ne mogu koristiti za dalju reprodukciju. Cilj ovog rada je da se prikažu klanični parametri trupa i randman sanske jaradi. Deset muške jaradi sanske rase je nasumično uzeto sa jedne farme. Jarad su hranjena po volji zamenom za mleko, koncentrat i seno lucerke su takođe dobijali po volji. Na 74 dan hrana im je bila uskraćena preko noći, imali su pristup vodi a 75 dana ujutru su transportovani na Institut za Stočarstvo u Zemun gde je obavljeno klanje i gde su uzete sve mere na trupovima. Statističke analize kao što je deskriptivna statistika (aritmetička sredina, standardna greška, standardna devijacija, koeficijent varijacije, minimum i maksimum) je odrađena u softveru Statistica 12. Pre klanja je izmerena živa masa (oko 20,35kg), a nakon klanja topli trup bez iznutrica (oko 10,56kg), masa organa (jetra 0,46kg; pluća 0,45kg ; srce 0,10kg; slezina 0,04kg; omentum 0,40kg; testisi 0,06kg; otpad 4,14kg; tanka creva 0,57kg; noge 0,75kg; koža 1,84kg). Prosečan randaman trupa je 51,85%. Može se zaključiti da iako sanska rasa služi za proizvodnju mleka, rezultati ovog rada pokazuju da sanska jarad imaju povoljne klanične parametre i dobar randman, ali su potrebna dalja istraživanja na ovu temu što je i predviđeno projektom Ministarstva nauke.

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SEROLOGICAL STUDY ON *BRUCELLA SPP.* AND SMALL RUMINANT LENTIVIRUS IN DAIRY GOATS IN VOJVODINA

I. Pusic[1], D. Lupulovic[1], J. Prodanov-Radulovic[1], M. Urosevic[2], Z.Grgic[1]

[1]Vetrinary Scientific Institute "Novi Sad", Rumenacki put 20, Novi Sad, Republic of Serbia
Corresponding author: ivan@niv.ns.ac.rs
[2]High school for agriculture "Futog", CariceMilice 2, Futog
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Abstract: The objective of this study was to determine seroprevalence of small ruminant lentivirus (SRLV), *Brucella abortus* and *Brucella melitensis* infections among dairy goats in South Backa and Srem region in Vojvodina Province. The other aim was to determine the risk factors associated with SRLV infection in investigated herds. A total of 971 serum samples were collected from 8 comercial dairy goat herds distributed in these two districts. All collected samples were examined for antibodies against *Brucella abortus* and *Brucella melitensis* by an competitive enzyme linked immunosorbant assay (cELISA), and all yielded negative result. Sera from 216 goats older than six months were tested for antibodies against SRLV using commercialy available ELISA. A total of 73 goats in six herds tested seropositive for SRLV with overal seroprevalence of 33% indicating that the infected herds are evenly spread throughout these two regions. The prevalence of positive animals within the herds ranged from 0 to 100% and the prevalence rate of seropositive goats increased with age. On the herd level import of goats, herd size and rearing with sheep were the most significant risk factors. The result of present study indicates that SRLV infection exists in the goat herds in Vojvodina Province. It also provides an overwiev about most important risk factors connected with the disease in the examined herds. Specific antibodies against *Brucella abortus* and *Brucella melitensis* infection were not detected.

Keywords: dairy goats, lentivirus, brucella, serology, prevalence

Introduction

Estimated goat population in Serbia is about 320.000, with a large number of non-commercial husbandries predominantly located in mountainous regions (*Zujovic et al., 2011)*. Interest for intensive dairy goat farming in Vojvodina Province has significantly increased in recent years. Despite groving population of

goats in intensive farming system, the milk production in most operations remains unsatisfactory. Among other factors, infectious diseases such as brucellosis or caprine arthritis-encephalitis (CAE), may contribute to impaired milk production, and represent a significant trade barrier. Small ruminant lentiviruses (SRLV), including ovine maedi-visna virus (MVV) and capine arthritis-encephalitis virus (CAEV), are considered genetically as a single pathogen (*Shah et al., 2004)*. Infections caused by SRLV are widespread in many countries and are responsible for significant economic losses (*Peterhans et al., 2004).* Disease usually takes years to develop and is progressive, causing chronic and inflamatory lesions in the brain, lungs, joints and mammary glands of sheep and goats (*Dawson, 1989*). Brucellosis is one of the most important bacterial diseases that can affect goat production and cause signifficant economic lossess. During the last decade in Vojvodina Province there were several outbreaks of caprine brucellosis (*Lalic et al., 2004)*, which is last time reported in 2005. The purpose of this study was to document the presence and to determine seroprevalence of CAEV and to elucidate risk factors conected to infection. The other aim was to determine brucella status of the investigated herds.

Material and Methods

During the 2008 samples were taken from 8 commercial goat farms situated in South Backa and Srem region of Vojvodina Province. The goat flocks were chosen according to production type and geographical area in order to represent both regions. The goats are predominantly Alpine, Saanen or Toggenburg breeds, and are all kept under intensive conditions for milk production. A total of 971 blood samples were collected in vacutainer tubes from the jugular vein of each goat. Blood samples were chilled and transported to the laboratory where they were centrifuged at 1000g for 10 minutes. Serum was stored in 1.5 ml Eppendorf tubes and stored at -20ºC for batch testing. In order to detect anti *Brucella sp.* agglutinins, all the sera were tested with Rose Bengal serum aglutination test (VZ «Zemun») and competitive ELISA (Brucella-AbC-ELISA, Swanowa, Sweden). Sera from 216 goats older than six months (which represents about 20% of the total number of goats in each herd) were evaluated for antibodies against SRLV using commercialy available ELISA (CAEV/MVV antibody test kit Chekitt, IDEXX, Netherlands).

Results and Discussion

Seventy- three goats out of 216 tested, were found seropositive to CAE virus infection. On the herd level 6 out of 8 tested herds presented at least one

seroreactive animal, revealing that infection is spread uniformly through two regions (Table 1.).

Table 1. Goat herds examined with ELISA for CAEV antibodies in two Vojvodina regions

Region	Number of herds	Positive	Percent (%)
South Backa	4	3	75
Srem	4	3	75
Total	8	6	75

Although seroconversion does not neccessary mean that the animal was clinically afected, CAE seem to be widespread amongst goat flocks from South Backa and Srem region, and probably represents an important factor that contributes to the sub optimal productivity. Additionaly, in herd 5 where the prevalence of CAE seropositive animals was amongst the highest (Table 2.), the clinical findings of encephalitis in kids 2-4 months old were strongly suggestive of CAE and caused high mortality rates.

Table 2. Prevalence of antibodies against CAEV in tested goats from eight herds

Flock Number	Number of animals tested	Positive	Percent (%)
1	59	3	5
2	12	0	0
3	40	32	80
4	10	10	100
5	27	24	88
6	8	3	37
7	24	1	4
8	36	0	0
Total	216	73	33

Virus isolation was not attempted. However, the detection of CAEV antibodies in goats is considered to be diagnostic for the infection (*Adams et al., 1983, Cutlip et al., 1992)*, and decreased birth weights, delayed weight gains and increased mortality before weaning have been demonstrated in offspring (*Peterhans et al., 2004).*The high overall seroprevalence within the most of herds under the study implied that infection might have been present for several years.Seroconversion may be delayed, ranging from a few weeks up to 2 years post infection, and is well documented (*Adams et al., 1983, Blacklaws et al. 2004)*. Herd seroprevalence in this study (75%) was higher than that reported in Jordan (23%), Great Britain (10%), Wales (57%), but lower than that estimated in studies conducted in Norway (86%), Australia (82%) (*Al-Quadahet al., 2006, Dawson and Wilesmith 1985, Nord K et al., 1998, Greenwood PL et al. 1995).* This finding

might be due to a small number of herds included in the study, but a higher seroprevalence was recorded where intensive husbandry is generally practiced (*Cutlip et al., 1992)*. Information about prevalence of SRLVs in the goat population are less updated, but no EU country can be considered to be SRLV infection free (*Peterhans et al., 2004)*.In this study, goats older than three years were more likely to be seropositive than younger goats. The observed association between the age and seroreactivity is consistent with findings in previous surveys (*Nord K et al., 1998, Al-Quadahet al., 2006)*. The animals that escaped infection in neonate period, are at permanent risk of contracting infection later in life through horizontal spread of disease. The main route of transmission between flocks appear to be ingestion of infected colostrum or direct contatact with infected animal (*Blacklaws et al. 2004)*.Epizootiological investigation in this study revealed that single most important risk factor for CAE infection at herd level was import of goats. In 5 of 6 herds where seropositive goats were detected, replacement goats and bucks were imported from three different European countries. The exchange of live animals across the national boundaries is considered to be an important cause of horizontal transmission of SRLV (*Blacklaws et al., 2004)*. In one herd (herd 8), where no seropositive animals were found there was no history of buying replacements from abroad. In herd No1 where three seropositive animals were detected, the most probable source of infection was rearing goats in a close proximity with sheep flock that was previously diagnosed with clinical MVV and pulmonary adenomatosis (*Pusic et al.,2008)*. Evidence of cross-species transmission is documented earlier (*Castro et al., 1999, Shah et al., 2004)* and can be experimentaly induced (*Peterhans et al., 2004)*. Recent sequence comparisions of natural isolates of SRLV from sheep and goats suggested that horizontal cross-species infection can occur and that it is common (*Roland et al., 2002)*.In herd 7 only one buck that was purchased few months earlier from infected flock tested positive. Live animal trading is considered a major risk factor in spread of SRLV infection between herds. Presence of seropositive bucks in a herd poses a risk for spreading the pathogen through direct contact between infected buck and uninfected does, although sexual route of transmission seems not to be well documented yet (*Blacklaws et al., 2004)*. Our data suggest that addition of new animals, contact with sheep flocks, herd size and stocking density are associated with high seropositivity to CAEV in intensive goat rearing. Out of 971 serum samples tested for anti- *Brucella sp.* aglutinins, neither one yielded a positive reaction. Occurence of brucellosis in goat population in Vojvodina during last decade was documented (*Lalic et al., 2004),* but since 2005 when 145 infected goats were culled there is no further evidence of seropositive animals in South Backa and Srem region, as our study also suggest.

Conclusion

The information obtained on seroprevalence in this study reveals that SRLV infections among goat herds in South Backa and Srem region are evident. It also indicates that in future more clinical outbreaks are to be expected, and goat breeders should be informed about the role of SRLV infection on animal welfare and potential economic losses. Carefully tailored eradication strategies are required to prevent dissemination of disease, and SRLV-free certified flocks shoud be the ultimate aim. We found no evidence of brucellosis infection in investigated goat farms, and present survaillance programe schould be continued.

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Serološko ispitivanje prisustva infekcije mlečnih koza lentivirusima i *brucella* vrstama u Vojvodini

I. Pušić, D. Lupulović,J. Prodanov-Radulović, M.Urosević, Ž.Grgi[1]

Rezime

Cilj istraživanja bio je da se ustanovi seroprevalencija infekcije lentivirusima i bakterijama iz roda *Brucella* (*Brucella abortus* i *Brucella melitensis*) u zapatima mlečnih koza na teritoriji Južnobačkog i Sremskog okruga u Vojvodini. Takođe, cilj je bio i da se ustanove najvažniji faktori rizika povezani sa pojavom infekcije lentivirusima malih preživara (SRLV) u ispitivanim gazdinstvima. Ukupno je pregledan 971 uzorak krvnog seruma koza sa 8 komercijalnih farmi koje su bile smeštene u ova dva regiona. Svi uzorci ispitani su kompetitivnom ELISA metodom na prisustvo specifičnih antitela protiv *Brucella abortus* i *Brucella melitensis* i svi su dali negativan rezultat. Krvni serumi 216 koza starijih od 6 meseci ispitani su na prisustvo specifičnih antitela protiv lentivirusa malih preživara ELISA testom. Od ukupno ispitanog broja uzoraka seropozitivna su bila 73 grla iz 6 zapata, sa prosečnom seroprevalencijom od 33%, a inficirani zapati bili su ravnomerno raspoređeni u oba regiona. Prevalencija seropozitivnih

jedinki unutar zapata kretala se od 0 do 100%, pri čemu je rasla sa starošću životinja. Kao najvažniji faktori rizika za pojavu infekcije SRLV u zapatu identifikovani su: uvoz koza iz inostranstva, veličina stada i odgajanje u kohabitaciji sa ovcama. Rezultati istraživanja ukazuju da infekcija lentivirusima na farmama koza u odabranim regionima Vojvodine, perzistira i predstavlja značajan problem. Isto tako, ukazuju na najvažnije faktore rizika koji su povezani sa prisustvom i raširenosti SRLV infekcije u pojedinačnim zapatima. Prisustvo infekcije vrstama *B. abortus* i *B. melitensis* na ispitivanim farmama koza nije ustanovljeno.

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EFFECTS OF RAPESEED MEAL AS PROTEIN SOURCE IN CEREAL-BASED DIETS ON LAMB PERFORMANCE

M. R. Yossifov

Institute of Animal Science Kostinbrod, Bulgaria
Corresponding author: m_vet@abv.bg
Original scientific paper

Abstract: This study investigates the potentional of using Bulgarian produced biodisel by-products as part of diets for lambs. The objective of the research was to evaluate the lambs' response on rapeseed (RSM) vs. sunflower meal (SFM) as protein supplements in cereal-based diets. Thirty lambs (Bulgarian Dairy Synthetic Population, 25-d of age, 10.8 kg initial body weight (IBW)) were allocated to one of two experimental diets in 92-d continuous trail. Diets were iso-nitrogenous (15 % CP and 7 % PDI), iso-caloric (0.7 FUG) and equal to calcium/phosphorus ratio. Final BW (FBW), total gain and average daily gain (ADG) were lower, but no significant – 24.5 and 25.8 kg (p=0.5), and 0.150 and 0.163 g/d (p=0.4) for cereal-based diets supplemented with SFM than RSM, respectively. Also, there were no significant differences in dry matter (DM) intake, varied between 719 – 737 g /d. Feed conversion and nutrient efficiency were more useful for lambs fed RSM, compared with SFM-based diet. In conclusion, replacing all SFM (30 %) and part of wheat (1.3 %) with RSM improved growth performance (FBW, ADG), feed conversion and nutrient efficiency.

Keywords: lamb, protein source, rapeseed meal, gain, feed conversion, performance

Introduction

Bulgarian farmers have to identify profitable feeding systems that meet consumer requirements. So, lamb producers had to meet a challenge of intensive indoor feeding systems for fattening lambs. Choicing suitable protein source, as a supplement of cereal-based diets, with high quality and low rumen degradability (*Webster and Povey, 1990*), has resulted in higher growth rates. Traditionally, sunflower meal (SFM) has been used as a protein supplement in Bulgarian lamb diets. The main factor for low-priced high-quality protein sources is usage of locally produced feeds as a counterpoise of imported protein sources.

Rapeseed is reliable high-protein energy crop, used as raw material in biodiesel industry. An oil extraction by-product is rapeseed meal (RSM). It's an eligible source of crude protein (30 – 40 %) with optimal amino acid profile (*Mansour et al., 1993*; *Newkirk et al., 2003*), especially methionine, lysine and cystine (*www.canolacouncil.org*). Additionally, protein degradability (RDP) varied 51 – 69 % (*Kendall et al., 1991*; *Woods et al., 2003a*).
A few studies were conducted on the usage of RSM as a part of lamb' finishing diets (*Liu et al., 1998*; *Wiese et al., 2003*; *Goopfert et al., 2006*) and no anyone with young lambs. Moreover, most of them found negative effect on lamb consumption and performance. Recommended optimal RSM inclusion levels in lamb diets are less than 10 % (*Goopfert et al., 2006*). Therefore, we undertaked serries of investigates (*Yossifov et al., 2011*) needed to evaluate the potential of including higher levels locally produced RSM and to investigate the productive effects. The objectives of this study was to evaluate the effects of 32 % rapeseed meal as protein supplement in cereal-based lamb diets, on the *diet utilisation* (dry matter intake (DMI), feed conversion (G:F) and nutrient efficiency (dry matter (DM), crude protein (CP), protein trully digestible in intestines (PDI) and net energy for gain (FUG) per 1 kg lamb gain) and *growth performance* (growth rate, final body weight (FBW), average daily gain (ADG), etc.).

Material and Methods

Experimental animals. The 99-d feeding experiment (7 + 92-d) was carried out at the Institute of Animal Science, Kostinbrod, BG, with thirty lambs (IBW 10.80 ± 3.19 kg, 25-d) of Synthetic Bulgarian Dairy Population (SBDP) from our own farm. They were weaned and randomly allocated (by weight, sex, day of birth, type of litter) into two dietary treatments (n=15) – control diet (CD) with SFM and experimental diet (ED) with RSM. Lambs were weighed (with 12 h withdrawal of feed or water) prior to feeding twice a month throughout experimental period (92-d), and to obtain initial (days 0, 1) and final (days 91, 92) live weights – in two consecutive days. Based on weights obtained on day 0, lambs were sorted by weight for assignment to one of two treatment diets (n=15).

Dietary Treatments. Experimental diets (table 1) were prepared using the ingredients described in table 2. The concentrate mixes containing SFM and RSM, as a protein source, were formulated to be iso-nitrogenous, iso-caloric and aligned in Ca, P and protein truly digestible in small intestines (PDI). Total rations (TR-s) were consisted of 29 % meadow hay (MH) and 71 % concentrate mix (as

Table 1. Experimental scheme

Forage		CD /with SFM/		ED /with RSM/	
		(g)	(%)	(g)	(%)
Roughage:					
Meadow hay		230.00	29.02	230.00	29.05
Concentrate mixture:					
Protein source		240.00	30.29	250.00	31.58
Wheat		150.00	18.93	140.00	17.68
Corn		150.00	18.93	150.00	18.95
Vitamin-mineral mix:*		22.46	2.83	21.69	2.74
Total	Roughage	230.00	29.02	230.00	28.77
	Concentrate mix	562.46	70.98	561.69	71.23
	Total ration	792.46	100.00	791.69	100.00

CD-control diet; ED-experimental diet; RSM-rapeseed meal; SFM-sunflower meal.
* Provided the following per kg of diet: manganese-60.0 mg, iron-1.3 mg, copper-1.0 mg, iodine-1.6 mg, zinc-60.0 mg, cobalt-1.0 mg.Vit.A-5000IU, Vit.D-2000 IU, Vit.E-10.0 mg.

fed basis) and was formulated to meet and exceed all nutrient requirements of fattening lambs (*NRC, 2007*). Diets contained 3 % supplement, which provided Ca (limestone) and vitamin-mineral mix (manganese-60.0 mg, iron-1.3 mg, copper-1.0 mg, iodine-1.6 mg, zinc-60.0 mg, cobalt-1.0 mg.Vit.A-5000IU, Vit.D-2000 IU, Vit.E-10.0 mg). Feed was offered twice daily - concentrate mix (offered at 8.00 and 14.00 h) and roughage (offered at 10.00 and 16:00 h) were fed separately throughout the experimental period. Feed intake was adjusted weekly to allow an excess of 5 % of their anticipated intake expressed as fed basis. Animals were provided *ad libitum* access to feed and water throughout the study.

Sample Collection and Analyses. Diet ingredients were sampled monthly and composited for analysis. Remainders were collected and weighed daily and analized twice a month. Samples were analyzed for DM by drying in a forced-air oven at 65^{o}C for 48 h and then 105^{o}C. Dried feed samples were ground to pass through a 1-mm screen and analyzed for Crude Protein (CP) (Kjeldahl Nx6.25), Ether Extract (EE), Crude Fibers (CF), Ash, Calcium (Ca) and Phosphorus (P) (*AOAC, 2002*).

Table 2. Chemical composition of the feedstuffs used in the experiment*

Feedstuffs	DM	Chemical composition (DM basis %)					
		CP	EE	CF	Ash	Ca	P
Roughage:							
Meadow hay	80.68	6.63	1.65	30.66	6.34	0.38	0.09
*Concentrate mixture**:							
Sunflower meal	84.62	36.55	1.37	21.96	7.14	0.41	1.04
RSM	85.19	34.42	2.76	12.39	6.78	0.60	0.87
Wheat	86.88	11.70	2.35	2.62	1.96	0.07	0.33
Corn	86.29	9.28	3.42	3.84	1.47	0.06	0.24

1 kg total ration provide:

	FUG	CP, %	PDI, %	Ca:P ratio
CD	1.1	16	11	2.19
ED	1.1	15	10	2.19

* All values except DM are on DM basis. DM- dry matter, CP- crude protein, EE- ether extract, CF- crude fiber, DDGSw- dried distillers' wheat grains with solubles; FUG- feed units for gain, PDI - protein truly digestible in small intestines, BPR- balance of protein in rumen.

Parameters. Dry matter intake (DMI) was calculated as (feed delivered /remainders collected) x % DM of the diet fed. Average daily gain (ADG), DMI, feed efficiency rate and gain:feed (G:F) were determined in each 15-d period which animal weights were obtained (per group).

Statistical Analysis. Feed intake and DMI /average per lamb/, average daily gain, gain efficiency and other parameters were analyzed using *MS Office 2007* and Student's t-test.

Results and Discussion

Diet composition. The chemical composition of the protein forages varied. Rapeseed meal is well-known protein source in many countries with high-productive animal husbandry, while there has been relatively little data on RSM as a feed in our country. Chemical analysis of rapeseed meal (RSM) was comparable with previous other our research (*Yossifov et al., 2011*). Content of CP varied from 38 – 46 %, EE from 1.0 - 3.0 %, CF from 10.0 - 24.0 %, phosphorus from 1.0 - 1.2 and calcium from 0.6 - 0.8 % (*www.canolacouncil.org*). In the current study, the nutrient content of the RSM was within the lower range of the reported values. SFM had similar content (as fed basis) with RSM (except for CF value). Approximately similar RSM's characteristics were observed by *NewkkirkPresntPpt.pdf*. On the contrary, relatively higher % was found by *Feedbase* (*2008*). Also significantly lower values were published by *NRC* (*2001*).

EE level (2.76 %) was in the range noted by www.countrywidefarmers.co.uk, but lower than the values by *Feedbase* (*2008*) and higher than *NewkirkPresntPpt.pdf* и *www.canolacouncil.org.* CF (12.39 %) content corresponded to reported by *Bell et al.,* (*2001*), but were lower than those found by *Feedbase (2008*).

According to experimental design, diets was equal in energy (net energy as FUG) and protein concentration (table 2). So, feeding value of diets was equal in net energy (1.1 FUG), crude protein (15-16 %) and PDI (10-11 %). Calcium:phosphorus ratio of the total ration was formulated to meet and exceed requirements of fattening lambs (*NRC, 2007*). The content of PDI was high (table 2) as a result of high level of protein degradable in rumen (RUP).

Dry matter intake. The feed intake (table 3) reported as g.day^{-1} was negligible affected among SFM and RSM treatments (736.77 vs. 718.65 g.d^{-1}) or 2.4 %. DMI of concentrate mix (C), roughage (R) and total ration (TR) presented as g.day^{-1} on DM basis (Fig. 1) were not different among the groups (CD vs. ED).

Table 3. Average daily intake of DM and nutrients[a]

Item		DM	Nutrients (DM basis %)			
			CP	PDI	BPR	FUG
Concentrate mix[b]:	CD	528.49	111.00	67.59	17.26	0.69
	ED	500.56	103.03	53.96	22.62	0.62
Roughage:	CD	208.28	13.81	13.33	-3.58	0.12
	ED	218.09	14.46	13.96	-3.75	0.12
Total ration:	CD	736.77	124.80	80.92	13.70	0.81
	ED	718.65	117.49	67.92	18.87	0.74
DMI (g/kg BW$^{0.75}$)			CD		ED	
			69.7		65.3	

a All values are on dry matter basis except dry matter;
b Without premix. DM- dry matter, CP- crude protein, PDI- protein, trully digestible in small intestines, BPR- balance of protien in rumen.

The hay (0.21 and 0.22 kg.d^{-1}), concentrate mix (0.53 and 0.50 kg.d^{-1}) and TR (0.74 and 0.72 kg.d^{-1}) intake (as DM basis (fig. 1)) were within near range between groups. So, RSM at 32 % dietary inclusion was not high enough to depress DMI. There were no significant (p=0.4) effects of diets on DMI expressed on a metabolic BW (BW$^{0.75}$) basis (table 3). The protein (CP, PDI, BPR) and net energy (FUG) intake from TR were differ in range between groups (6, 16, 38 and 9 %).

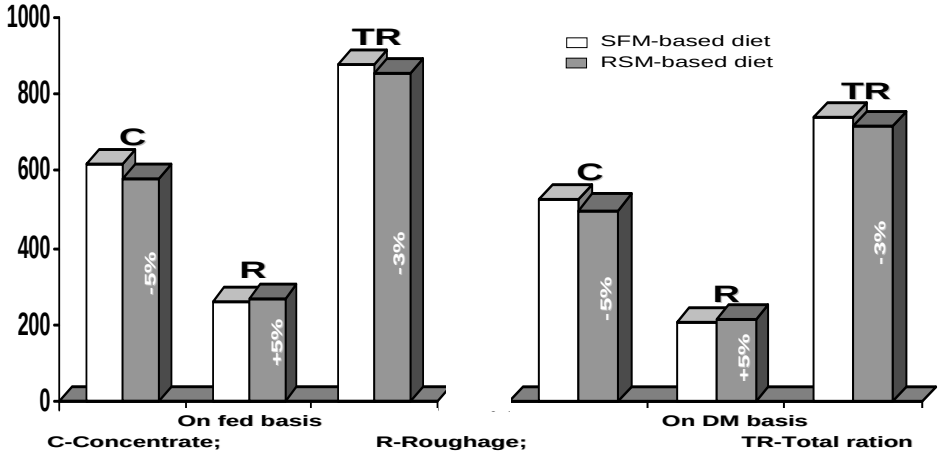

Figure 1. Average daily intake (ADI) of concentrate mix, roughage and total ration on fed and DM basis (g):

The intake of RSM-based diets in the current study agrees with the reported data by other authors (*Liu et al., 1999*; *Mandiki et al., 2006*) quite the contrary of *Vincent et al.* (*1990a*) and Wiese et al. (2003). First found lower and litter – significantly higher DMI in finishing lambs fed RSM, which replaced a portion of grain and protein source in the diet.

There were differences among treatments (Fig. 2) in values about intake of DM (4.94 vs. 4.42 kg), CP (0.84 vs. 0.72), PDI (0.54 vs. 0.42) and net energy as FUG (5.43 vs. 4.55) per 1 kg (PDI and FUG was calculated on literature data). It was observed benefits from ED in conversion of DMI, CP, PDI and FUG (- 11, 14, 22 and 16 %) per 1 kg gain compared with CD.

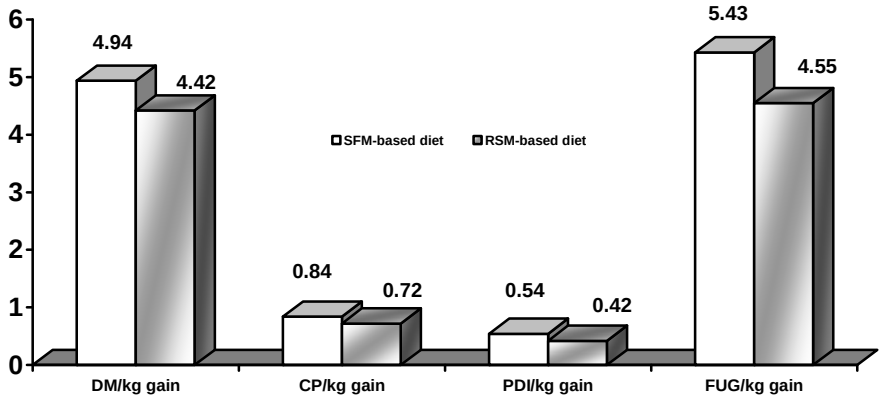

Figure 2. Nutrient efficiency (kg/kg gain) of fattening lambs fed SFM vs. RSM as protein source:

Growth performance and gain efficiency. The initial BW (IBW) didn't differ (10.8 kg ± 3.2) among treatments (table 4). The final BW (FBW) was higher

Table 4. Average daily gain (ADG) and Gain efficiency (G:F) of fattening lambs fed different protein source (SFM vs. RSM) /n=15 x±SD/

Item	Diet		Significance (P)
	SFM	RSM	
Initial BW (kg)	10.73 ± 3.23	10.87 ± 3.26	NS
Final BW (kg)	24.47 ± 5.89	25.83 ± 6.37	0.5
ADG ($g.day^{-1}$)	149 ± 36.6	163 ± 50.7	0.4
G:F ratio (ADG:DMI)	0.203	0.226	0.4

* Means within rows having superscript are significantly different at $p<0.05$. SFM- sunflower meal, DDGSw - dried distilers' wheat grains with solubles, BW- body weight, ADG- average daily gain, DMI- dry matter intake, G:F- gain/forage.

(p=0.50) for lambs fed RSM-based diet (25.83 kg) vs. control diet (24.47 kg). Lambs fed RSM-based diet had unsignificantly higher ADG for whole experimental period. The ADG (table 4) was higher (p=0.40) for ED (163 $g.d^{-1}$) compared with CD (149 $g.d^{-1}$). Our data corresponded to these reported by *Wiese et al.* (*2003*).

The feed conversion (DMI/ADG) was also more effeicient for lambs from ED (0.23) vs. CD (0.2). Nevertheless, the corresponding values (+ 11 %) for gain efficiency among treatments were not significant (p= 0.4).

In agreement with our previous work (*Yossifov et al., 2011*) and findings by *Wiese et al.* (*2003*), rapeseed meal seem to be a good protein forage in lamb diets, by reason of its high content of rumen undegradable protein (RUP).

Conclusion

Supplementing cereal-based lamb's diets with rapeseed meal improved growth performance, feed conversion and nutrient efficiency. Inclusion of RSM has no impact effect on DMI (+ 2.4 %) but improve performance (p=0.5) when included at 32 % of diet DM. It was amended the ADG by 9 % and feed conversion by 11 %. The results of this study indicate that RSM can completely replace sunflower meal (30 %) and a portion of wheat (1.3 %) from the diet when feeding fattening lambs without any compromise to performance (FBW, ADG), feed conversion and nutrient efficiency.

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Efekti korišćenja sačme uljane repice kao izvora proteina u obroku na bazi žitarica na proizvodne rezultate jagnjadi

M. R. Yossifov

Rezime

Ova studija istražuje potencijal korišćenja nusproizvoda iz proizvodnje biodizela u Bugarskoj, kao dela obroka za ishranu jagnjadi. Cilj istraživanja bio je da se proceni reakcija jagnjadi na obrok sa uljanom repicom (RSM) prema suncokretovoj sačmi (SFM) kao i proteinskim dodacima u ishrani na bazi žitarica. Trideset jagnjadi (bugarska mlečna sintetička populacija, 25-D starosti, 10.8 kg početne težine (ibv)) izdvojeno je za jedan od dva eksperimentalna tretmana ishrane u 92-dnevnom kontinuiranom ogledu. Obroci su IzO-azotni (15% CP i 7% PIO), IzO-kalorijski (FUG 0.7) i jednaka je odnos kalcijum/fosfor. Završna TM (FBV), ukupan prirast i prosečan dnevni prirast (ADG) su bili niži, ali ne značajno - 24,5 i 25,8 kg (p = 0,5), i 0,150 i 0,163 g / d (p = 0.4) za obroke na bazi žitarica dopunjenih sa SFM nego RSM, respektivno. Takođe, nije bilo značajne razlike u suvoj materiji (DM), unos je varirao između 719 - 737 g / d. Konverzija hrane i efikasnost nutrijenata su bili korisni za jagnjad hranjena RSM, u poređenju sa SFM-obrokom. U zaključku, potpuna zamena SFM (30%) i dela pšenice (1,3%) sa RSM poboljšala je performanse rasta (FBV, ADG), konverziju hrane i efikasnost korišćenja hranljivih materija.

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THE EFFECT OF INTROGRESSED ROMANOV BREED ON LITTER SIZE IN IMPROVED JEZERSKO-SOLČAVA BREED

A. Cividini[1], M.Kastelic[2]

[1] University of Ljubljana, Biotechnical faculty, Department of Animal Science, Groblje 3, 1230 Domžale, Slovenia
[2] Jata – Emona, Agrokombinatska 84, 1000 Ljubljana, Slovenia
Corresponding author: angela.cividini@bf.uni-lj.si
Original scientific paper

Abstract: The Improved Jezersko-Solčava breed (JSR) was established in 1980's with introgressed Romanov (R) breed into the Slovenian autochthonous Jezersko-Solčava (JS) breed with the aim to improve fecundity. Significant improvement was achieved in litter size (1.6 liveborn lambs per parity) compared to the JS breed (1.2). This improvement was stable up to the year 2006, when a decrease started. Generally, the level of productivity decreased in JSR population due to the economic conditions. In addition, the increased use of JS rams in JSR flocks was observed in selection program data base. These factors were studied via the genealogical analysis of breed proportions and seasonal trends and their effects on litter size. Data was analysed by the GENMOD procedure for the categorical response variable of litter size per parity and cumulative number of live born lambs up to n^{th} parity. The log-linear model included flock as a fixed effect and linear regression of birth year of ewe and the percentage of R breed in JSR breed. In the period from 1995 to 2009, the average percentage of R breed in the JSR population decreased from 49.16% to 44.65%. There was a correlation between litter size and percentage of R breed. The cumulative number of liveborn was affected by the percentage of R breed from the 2^{nd} to 7^{th} lambing. The cumulative number of liveborn lambs was also influenced by flock. It can be concluded, that decreased percentage of R breed significantly reduced the litter size in JSR ewes.

Key words: Improved Jezersko-Solčava breed, fertility, litter size, percentage of Romanov breed

Introduction

Improved Jezersko-solčava breed (JSR) was established by crossbreeding of Slovenian autochthonous Jezersko-solčava (JS) breed with Romanov (R) breed. The aim of the planned crossing was to improve the fertility of the new established

breed, especially to increase the litter size. Litter size is defined as a number of lambs born per ewe per lambing. The improvement of litter size was of great economic interest in JS meat sheep flocks in Slovenia in early 1980's when the first crossbreeding was done. The new breed fulfilled expectations and the breed was well accepted by breeders. Today, JSR is traditional sheep breed and the most important meat breed in Slovenia with its own breeding program.

The first crossing of JS ewes with R ram was done in controlled flock where the average litter size was 1.25 born lambs per litter *(Zagožen, 1983).* Four years later the flock was nearly complete with crosses of original ewes and Romanov rams. The average litter size was then 2.07 in total 388 lambings. The flock consisted mostly of F1 crosses, where an additional effect of heterosis on litter size was expected which disappeared in next generations *(Zagožen, 1983).*

In year 1998 there was 3387 lambings in controlled JSR population, where the average litter size was 1.54 born lambs *(Cividini et al., 2000).* The average litter size was increasing to the year 2003 when it reached an average of 1.62 born lambs at 3472 lambings *(Cividini et al., 2005).* After 2003 the average litter size in JSR population decreased and in 2008 reached 1.51 born lambs per litter *(Zajc et al., 2010)*. Romanov rams were often used in the formation of synthetic prolific breeds of sheep. The most known are synthetic INRA 401 *(Cruston, 1996)* and Spanish "Salz" *(Maria and Ascaso, 1999).* The Spanish "Salz" line produced 0.25 more lambs per litter compared to the local breed *(Maria and Ascaso, 1999)*.

Synthetic breeds with Romanov genes, like INRA 401, Salz or JSR, have shown an increase of litter size. Litter size of final synthetic breeds was smaller than litter size of F1 generations *(Zagožen, 1983; Maria and Ascaso, 1999).* This was expected because of lower heterosis in generations after F1. That phenomenon, known from theory of quantitative genetics does not explain the permanent decreasing of litter size after twenty years of breeding of Slovenian JSR breed. Sheep farms in Slovenia are almost predominantly characterized by low input production systems, which are present in ¾ of all farms and some 3/5 of the total animal population *(Šalehar et al., 2003)*. During the last few years the management of Slovenian sheep flocks has changed and also the economic environment in which the JSR was well established has been changed too. The paradigm of low input farming, supported with governmental subsidies, was generally well accepted by small sheep farmers. Consequently, the purebred JS rams were used in JSR flocks to decrease the litter size.

The aim of this study was to find out, if permanent decreasing of litter size in JSR population is affected by changed management of JSR animals or also by decreased genetic influence of R breed.

Material and Methods

In this study the data for JSR breed were obtained from the Republic Data Base for Selection of Small Ruminants, between 1995 and 2009. The data collection includes pedigree of animals and production data. Although the origin of animals was exactly known, the breed of animals was often determined subjectively by control assistant. The litter size measures were analysed according to the percentage of R gene in JSR breed and year of lambing (parity). The observed litter size was at 1^{st}, 2^{nd}, … and 8^{th} parity. The cumulative number of live born lambs was calculated from 1^{st}-2^{nd}, 1^{st}-3^{th}, 1^{st}-4^{th}, 1^{st}-5^{th}, 1^{st}-6^{th}, 1^{st}-7^{th}, 1^{st}-8^{th} parity. The number of ewes observed (N) for litter size and cumulative production of lambs was 20493 in total. Because the litter size and cumulative number of live born lambs as traits belong to the count data, Poisson error structure was assumed for litter size and cumulative production of lambs.

Data was analysed by the GENMOD procedure (Generalised Linear Models) in *SAS/STAT statistical software, version 9.2, 2001),* which allows distributions other than a normal one. The log-linear model (1) for litter size and for cumulative number of live born lambs (Y_{ij}) included flock (farm management) (F_i) as fixed effect. The linear regression coefficient (b_1) of the birth year of ewe (X_{ij}), and the linear regression coefficient (b_2) of percentage of R gene in JSR ewes (R_{ij}) were included in the model, where e_{ij} was the error of any individual measurement, distributed according to Poisson distribution.

$$Y_{ij} = \mu + F_i + b_1\left(X_{ij} - \overline{X}\right) + b_2\left(R_{ij} - \overline{R}\right) + e_{ij} \qquad (1)$$

Results and Discussion

The average parity, average litter size and average percentage of R breed in JSR ewes according to the year of lambing are presented in Table 1.

Table 1. Average parity, average litter size and average percentage of R breed (% R) in ewes according to the year of lambing.

Year of lambing	N	parity	litter size	% R
1995	1185	3.29	1.60	49.16±4.65
1996	1642	3.41	1.60	48.80±5.13
1997	2419	3.53	1.56	47.82±6.74
1998	3490	3.45	1.59	47.43±6.99
1999	4753	3.63	1.55	47.77±6.45
2000	4784	4.13	1.55	47.64±6.83
2001	4861	4.51	1.58	47.25±7.29
2002	4667	4.86	1.56	47.34±7.91
2003	4971	4.98	1.59	46.96±7.98
2004	4817	4.90	1.56	46.63±8.65
2005	4656	4.98	1.56	45.87±9.01
2006	4691	5.35	1.52	45.98±8.58
2007	4748	5.31	1.50	45.23±9.51
2008	3989	5.49	1.50	44.86±9.61
2009	1014	5.53	1.52	44.65±9.71

Litter size of JSR ewes constantly decreased with advance in parity (Table 1). In year 1995 the litter size of JSR ewes was 1.6 in 2007 it dropped to 1.5. These results are contrary to those observed by *María and Ascaso (1999)*, where litter size increased with advance in parity. *María and Ascaso (1999)* observed the largest litter size in ewes of four breeds in their fourth parity (1.59) with a more evident increasing litter size trend in Romanov ewes. Normally, it is expected that the litter size improves with advance in age through the increased ovulation rate, uterine capacity and other maternal traits affecting the reproductive efficiency of the ewes *(Fahmy, 1990).* In present study, the litter size of JSR ewes decreased mostly due to the decreasing percentage of R breed through the lambing years. In year 1995, the average percentage of R breed in JSR ewes was 49.16 % with standard deviation of 4.65 (coefficient of variability, CV, 9.46 %). Subsequently, the level of R genes decreased to 44.65 % with standard deviation of 9.71 (CV 21.75 %) in the year 2009. The decreasing trend of the percentage of R genes in JSR population can be explained by including purebred JS rams into JSR flocks. Later the offspring with lower proportion of R genes were officially declared as JSR animals. The rams were tested on growth traits and introduced in purebred JSR flocks. Moreover, the flock management was changed with the introduction of negative selection on litter size in JSR flocks as twins or triplets were not desired in flocks any more. Only really interested, professional breeders of JSR breed with

the high productive animals developed good management and selection on larger litter size.

The Spearman correlation coefficients (not shown) confirm positive relations between the percentages of R genes in JSR breed and litter size. The estimated partial correlation was low (0.0469) but positive. One – way statistical evaluations cannot exclusively confirm the effect of percentage of R genes in JSR breed. One source of variance can be overlapped with other effects which change with the same tendency, for example the percentage of R genes in JSR, and the quality of management. In Table 2, the source of variability and Wald statistics for distribution of litter size by parity and cumulative number of live born lambs up to n^{th} parity is shown.

Table 2. Source of variability, and Wald statistics for distribution of litter size by parity and cumulative number of live born lambs up to n^{th} parity

			Effect			
	Flock (F)		Birth year of ewe (X)		% R breed (R)	
Litter size Parity	df	P	df	P	df	P
1	287	0.9990	1	0.1238	1	0.0063
2	282	0.3450	1	0.4889	1	0.0479
3	264	0.2745	1	0.2819	1	0.1373
4	243	0.4653	1	0.1142	1	0.0702
5	235	0.0523	1	0.3526	1	0.5774
6	214	0.0346	1	0.2407	1	0.2368
7	201	0.5536	1	0.7302	1	0.6910
8	183	0.6031	1	0.7990	1	0.2691
Total lamb production up to n^{th} parity						
2	264	<0,0001	1	0,1481	1	0,0015
3	245	<0,0001	1	0,0497	1	0,0036
4	219	<0,0001	1	0,0339	1	0,0016
5	194	<0,0001	1	0,0404	1	0,0058
6	177	<0,0001	1	0,0476	1	0,0157
7	159	<0,0001	1	0,0575	1	0,0575
8	138	<0,0001	1	0,1296	1	0,0600

The litter size by parity was not affected by flock except the litter size in 6^{th} parity. Birth year of ewe was not a significant factor affecting the litter size. However, litter size was influenced by the percentage of R breed in JSR breed in the first and second parity.

Total lamb production up to n^{th} parity was affected by flock with high probability ($P<0.0001$). It was expected, that the management on farm influenced the fertility, especially because no standard production system can be found in Slovenia. Total lamb production up to 3^{th}, 4^{th}, 5^{th} and 6^{th} parity was influenced by birth year of ewe. Total lamb production up to 7^{th} and 8^{th} parity was not affected by birth year of ewe, probably due to culling the ewes with low productivity before 7^{th} parity. The total lamb production per ewe between parities 2 and 6 was also influenced by the percentage of R breed. In the future it is necessary to reduce the percentage of JS breed in JSR population on the expected value of 50 %. The producers, which prefer smaller litter size, should be treated separately in breeding program of JSR. Otherwise, the productivity of JSR breed will generally be decreased.

Conclusion

The results of this study showed that the percentage of R breed in JSR population decreased from 49.16% in 1995 to 44.65% in 2009. The litter size per parity was correlated with percentage of R breed in JSR ewes, so the decreasing was obvious. However, the percentage of R genes in JSR ewes influenced only litter size in the first and second parity. But the cumulative number of liveborn up to parities 2, 3, 4, 5 and 6 was influenced by the percentage of R genes in JSR breed. The flock (farm management) highly influenced ($P<0.0001$) the total lamb production. Decreasing trend of litter size in JSR population was not only the consequence of changed farm management but also the consequence of decreased percentage of R breed in JSR population.

Efekat introgresirane romanov rase na veličinu legla u oplemenjenoj jezersko-solčavskoj rasi

A. Cividini, M. Kastelic

Rezime

Poboljšana jezersko-solčavska rasa (JSR) je tradicionalna slovenački ovaca rase. Rasa potiče od rodnog jezersko-solčavska (JS) rase koja je poboljšana korišćenjem Romanov rase da bi dobili rasu ovaca sa dobrim karakteristikama majki i veću veličinu legla. Poboljšanje je bio stabilno do 2006 godine, kada je počelo smanjenje veličine legla. U odabiru baze podataka programa povećana upotreba JS ovnova u jatima JSR je primetno. U ovom radu su uključeni podaci iz baze podataka za izbor programa JSR rase između godina 1995 i 2009.

Prikupljanje podataka sadrži pedigre životinja i proizvodnih podataka. Osobine veličinu legla su analizirani prema procentu R gena u JSR rase i godine jagnjenju (pariteta). Zato veličina legla i kumulativni broj živorođene jagnjadi kao osobina pripada uključenim podacima, Poisson struktura greška je pretpostavljena za veličinu legla i kumulativnu proizvodnja jagnjadi. Podaci su analizirani u GENMOD postupku za dobijanje kategoričnog odgovora na promenljivu veličinu legla po paritetima i kumulativni broj živorođene jagnjadi do n-tog pariteta. Log-linearni model uključivao je i stado kao fiksni efekat i linearne regresije godine rođenja ovce i procenat R rase u JSR rase.

U periodu od 1995 do 2009, prosečan procenat R rase u JSR populaciji opao je sa 49,16% na 44,65%. Došlo je do korelacija između veličine legla i procenta R rase. Kumulativni broj živorođene je bio pod uticajem procenta R rase od 2. do 7. pariteta. Kumulativni broj živorođene jagnjadi je bio pod uticajem i stada. Može se zaključiti, da je smanjenje procenta R rase značajno umanjilo veličinu legla JSR ovaca.

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CARCASS CHARACTERISTICS AND SOME MEAT QUALITY PARAMETERS OF KIDS OF BALKAN GOAT BREED SLAUGHTERED AT DIFFERENT WEIGHTS

M. Žujović, N. Stanišić, Z. Tomić, N. Maksimović, Z. Bijelić, V. Mandić

Institute for Animal Husbandry, 11080 Belgrade, Republic of Serbia
Corresponding author: nikola0135@yahoo.com
Original scientific paper

Abstract: The effect of pre-slaughter weight on carcass characteristics and composition, and quality of kid meat, was investigated. Trial was carried out on 18 kids of Balkan goat breed, slaughtered at body weight of: 10, 15 and 22 kg. Heavier kids had lower meat yield/dressing percentage of warm and cold carcass compared to lighter kids, and higher share of fat tissue in carcass. Chill loss was the lowest (4.00%) in kids weighing 22 kg, which is most likely the consequence of thicker subcutaneous fat tissue. Heavier kids had higher share of first category parts (leg and loin). Increase of weight at slaughtering influenced the increase of share of intramuscular fat in samples of *longissimus dorsi* muscle, which varied from 1.51% in kids weighing 10 kg to 2.09% for kids weighing 22 kg ($p<0.05$). Technological meat quality was better in heavier kids, with greater water binding capacity and lower cooking loss. Results obtained in this trial show positive effect of the increase of weight at slaughtering on carcass characteristics and meat quality.

Key words: Kids, weight at slaughter, carcass characteristics, meat quality.

Introduction

In the last decade, the number of goats has increased globally, even in countries with developed economies (*Morand-Fehr et al., 2004*), primarily for the production of milk, and then the meat. Increased interest in goat meat is primarily because goats have very little subcutaneous fat and intramuscular fat (*Kieton, 1988; Colomber-Rocher et al., 1992*), and lower fat intake through food is one of the important factors leading to reduction of risk of cardiovascular diseases (*Park et al., 1991; Giese, 1992; Pfeuffer, 2001*). In comparisons of different types of meat it was concluded that goat meat and kid meat in particular, has good nutritional properties and in this respect is considered as one of the most valuable types of meat (*Gibson, 1998)*.

In Serbia, the goat production before the Second World War constituted an important branch of animal husbandry in mountainous areas. However, the Law prohibiting the keeping of goats in year 1953 had a negative impact on the number of goats and production of quality goat products (milk and meat). From the seventh decade of the twentieth century, there is increasing interest in growing of goats, especially in areas where the conditions for the rearing of other ruminants are not so favourable, but also in areas with developed agricultural production, such as Morava district and province of Vojvodina. Number of goats is on the increase and goats for slaughter are now appearing on the market, mostly light (8-12 kg) and rarely slightly heavier animals (above 15 kg). Much of the goat population in the mountainous area of Serbia is the domestic Balkan goat, red to rufous colour with varieties of black, white and spotted goat *(Žujović et al., 2008, 2009)*. The domestic Balkan goat is predominantly reared in hilly-mountainous regions, and to a somewhat lesser degree in lowlands of Serbia, with predominantly extensive and semi-intensive rearing systems.

The objective of this trial was to study the characteristics of kids of Balkan goat breed, indigenous goat breed with the pre-slaughter mass of 10, 15 and 22 kg. Emphasis was placed on examining slaughter traits, carcass composition and important meat quality parameters.

Material and Methods

The trial was carried out in the Institute for Animal Husbandry (Belgrade, Serbia), on 18 kids of the Balkan goat breed, that were slaughtered at different live weight: 10 (n=6), 15 (n=6) and 22 kg (n=6). After weaning, all kids were reared under an intensive system of management and fed with grass hay and concentrate. Hay consisted of grass mixture (Festucetum vallesiacaeassociation) which comprises 57 species of uniform quality with regard to content of crude protein (7.60–9.53%) and crude fibre (27.49–29.55%). Composition of the concentrate used in the feeding was as follows: proteins (16.0%), moisture (13.5%), fibre (8.0%), ash (8.0%), calcium (0.8–1.0%), phosphorus (0.5–0.7%) and sodium (0.2–0.3%), with an addition of vitamins and minerals. Feeding was *ad libitum* and in groups until slaughter.

After reaching the specifed body weight, all animals were transported to the slaughterhouse. They were denied feed 12 h prior to slaughtering, but they had free access to water. Animals were electrically stunned and slaughtered according to standard commercial procedures. After the removal of skin and head, front and rear legs and evisceration, carcasses were placed in cold storage at 4ºC for the next 24 hours. One hour after slaughtering and primary processing of the carcass the weights of hot carcass (HCW), offals (heart, lungs, liver, kidneys, spleen, rumen,

abomasum, small and large intestines), skin and head, were recorded. Subsequent to cooling the weight of cold carcass (CCW) and weight of total fat tissue in the carcass (pelvic and kidney fat) were determined and the dressing percentage (DP) and chilling loss were calculated.

After chilling, the carcasses were split along the vertebral column into two halves, and the left side of each carcass was divided into seven anatomical regions: leg, loin section, shoulder, neck, back, chest with fore thigh and second thigh, using a standard technique.

Samples of the *M. longissimus dorsi* from the loin were taken from cooled carcasses. Water binding capacity was determined according to the method of *Grau et al. (1953)* and pH value of meat was measured by insertion of pH meter with combined electrode HI 83141 (Hanna Instruments, USA) using a muscle sample collected 24 hours postmortem. All samples were analysed for basic chemical composition: quantity of water, quantity of intramuscular fat, amount of protein and amount of mineral substances (ash) *(AOAC, 1990)*. The content of total pigments was determined according to the method of Horsney *(Bunning and Hamm, 1970)*.

The loss of weight during cooking (cooking loss) in muscle samples was determined based on difference in weight of one piece of meat (3×4×1.5 cm) before and after cooking (100ºC/10 min) and expressed in percentage. Muscle samples used for determination of cooking loss were cut into 1×1 cm pieces in the direction of muscle fibres. Tenderness of meat, expressed as shear force, was measured on the Volodkevich apparatus *(Volodkevich, 1938)*. Higher recorded values represented higher shear force, i.e. tougher meat.

In order to determine the effect of live weight on carcass and meat quality characteristics, a single-factor analysis of variance was performed using Statistica 7 software (StatSoft, USA). If the effect of live weight at slaughter was found significant, t-test was used to evaluate the significance of difference. All results in tables are expressed as arithmetic mean ± standard deviation.

Results and Discussion

In Table 1**,** the age and main characteristics of carcass, as well as offals, skin, head and fat tissue are presented. Mass of warm and cold carcass have increased as expected, whereas the dressing percentages/meat yields of warm and cold carcass have decreased with the increase of pre-slaughter mass of kids ($p<0.05$). In the study of the effect of age (from 56 to 166 days) on some slaughter parameters of Alpine breed kids, *Fehr et al. (1975)*, established that older kids, i.e. kids of higher body mass, had lower dressing percentage/meat yield compared to lighter kids. *Van Niekerk and Casey (1988)* report that significant differences

between goat genotypes with regard to dressing percentage depend greatly on the weight of offals and parts which are removed during slaughter. In this trial, the proportion of offals and head decreased with the increase in the weight at slaughter ($p<0.05$). Warm carcass dressing percentage/yield was the highest in kids slaughtered on reaching a mass of 10 kg (60.31%) and it was significantly different in comparison to the kids with the pre-slaughter mass of 22 kg (55.80%). Cold carcass yields did not differ significantly between the groups of kids. Similar values for dressing percentage of the warm carcass in two groups of kids of Serbian White breed, slaughtered at the age of 70 days, are reported by *Žujović et al. (2008)*. In the research carried out by *Gruszetski et al. (1997)* on 35 kids of White improved breed reared in Poland, slaughtered with body mass of 20 kg, the dressing percentage of cold carcass was 43.79%, which is similar with our results.

It is known from literature data that thickness of subcutaneous fat tissue has a very considerable impact on chilling loss *(Stanišić et al., 2012)*. This statement is confirmed by results obtained in this study, considering that kids with greater recorded mass of total fat tissue (22 kg group), had lower chilling loss (Table 1).

Table 1. Age, carcass characteristics and the share of offal, fat tissue, skin, and head of kids of different pre-slaughter masses

	10 kg	15 kg	22 kg
Age (days)	$61.50^{a} \pm 4.75$	$82.00^{b} \pm 8.11$	$136.00^{c} \pm 9.74$
HCW[1] (kg)	$6.16^{a} \pm 0.20$	$8.81^{b} \pm 0.71$	$12.45^{c} \pm 0.64$
DP[2] HCW (%)	$60.31^{b} \pm 3.30$	$58.16^{ab} \pm 2.14$	$55.80^{a} \pm 2.87$
CCW[3] (kg)	$4.48^{a} \pm 0.55$	$6.60^{b} \pm 0.57$	$9.56^{c} \pm 0.63$
DP CCW (%)	43.79 ± 1.20	43.54 ± 1.59	42.84 ± 1.15
Chill loss (%)	$4.66^{b} \pm 0.15$	$4.49^{b} \pm 0.22$	$4.00^{a} \pm 0.25$
Offals (%)[4]	$11.19^{b} \pm 0.65$	$10.02^{b} \pm 0.58$	$8.63^{a} \pm 0.29$
Total fat tissue (%)[4]	$1.93^{ab} \pm 0.72$	$1.81^{a} \pm 0.58$	$2.14^{b} \pm 1.06$
Skin (%)[4]	8.05 ± 0.96	7.52 ± 1.76	7.72 ± 1.62
Head (%)[4]	5.44c ± 0.64	4.62b ± 0.76	4.03a ± 0.16

[1] Hot carcass weight;
[2] Dressing percentage;
[3] Cold carcass weight;
[4] Calculated in relation to slaughter live weight;
[a, b, c] Means within rows bearing different letters are significantly different at $p<0.05$.

The share of the main parts of carcass sides differed significantly between the analysed groups of kids (Table 2). The highest share of the leg was found in the

kids of pre-slaughter mass of 22 kg (27.30%) and was statistically significantly higher compared to kids with pre-slaughter weight 10 kg. The share of loin had a similar trend and in kids with pre-slaughter mass of 22 kg it was about 5% higher than in kids weighing 10 kg ($p<0.05$). The share of the back was approximately the same between kids slaughtered on reaching 10 and 15 kg weight, but significantly higher compared to kids with pre-slaughter mass of 22 kg. The share of shoulder did not differ significantly between groups of analysed kids.

Table 2. Share of main carcass side parts in Balkan goat kids slaughetered at different weihts

(%)	10 kg	15 kg	22 kg
Leg	$22.31^{a} \pm 0.55$	$25.92^{b} \pm 0.92$	$27.30^{b} \pm 1.72$
Loin	$9.21^{a} \pm 0.39$	$9.79^{ab} \pm 1.01$	$10.20^{b} \pm 1.50$
Shoulder	18.97 ± 1.37	20.65 ± 1.34	21.07 ± 2.76
Back	$9.72^{b} \pm 1.14$	$9.48^{b} \pm 1.78$	$8.06^{a} \pm 1.79$
Neck	10.00 ± 2.05	8.39 ± 2.03	8.94 ± 1.32
Chest with fore thigh	$19.22^{b} \pm 1.15$	$17.93^{a} \pm 1.14$	$18.37^{b} \pm 2.07$
Second thigh	$8.48^{c} \pm 1.05$	$7.29^{b} \pm 0.69$	$5.58^{a} \pm 0.26$

$^{a, b, c}$ Means within rows bearing different letters are significantly different at $p<0.05$.

In Table 3, the basic chemical composition and some parameters of technological quality of samples of *longissimus dorsi* muscle are presented. Content of intramuscular fat increased statistically significantly with the increase of weight, i.e. age of kids. These data coincide with the results of *Bruwer et al. (1987)* and *Stankov et al. (2002)*. Share of proteins and ashes showed no significant differences between groups of kids, which coincides with results obtained by *Marichal et al. (2003)*.

pH value of meat, 24 hours subsequent to slaughtering, showed no significant differences between groups and ranged around 5.70, which is in accordance to results stated by *Stankov et al. (2002)* for kids at the age of 2 to 6 months. *Dhanda et al. (1999)* stated that range from 5,5 to 5,9 of final pH value is considered optimum for high quality goat meat.

Water binding capacity ranged from 9.77 to 11.42 cm^2 and more favourable values were determined in heavier kids (Table 3). Same conclusion was also made by *Stankov et al. (2002)*, who stated that water binding capacity increased with increase of age of kids.

The highest pigment content (93.10 ppm) was determined in meat obtained from kids of the 22 kg group and this value decreased significantly with the

decrease of pre-slaughter weight ($p<0.05$). Similar conclusion was made also by *Dhanda et al. (2003)*.

Shear force of *longissimus dorsi* muscle increased with the increase of kid weight and ranged from 12.09 to 18.15 kg. These values were high compared to results obtained by *Dhanda et al. (1999)*. Decrease of meat tenderness (higher values of shear force) with the increase of weight/age of kids was reported in work of *Dhanda et al. (2003)* and *Marichel et al. (2003)*. Available literature data pertaining to meat tenderness and shear force values indicated that it mainly doesn't have high degree of tenderness, even after longer maturation period *(Schönfeldt et al., 1993)*.

Table 3. Chemical and technological characteristics of *longissimus dorsi* muscle in kids depending on pre-slaughter weight

	10 kg	15 kg	22 kg
Chemical characteristics			
Water (%)	77.67 ± 2.40	76.58 ± 3.47	75.72 ± 4.77
Fat (%)	1.51^{a} ± 0.09	1.94^{b} ± 0.10	2.09^{b} ± 0.12
Protein (%)	19.63 ± 0.26	20.34 ± 0.37	21.05 ± 0.26
Ash (%)	0.96 ± 0.19	1.11 ± 0.12	1.09 ± 0.16
Technological characteristics			
pH 24	5.69 ± 0.25	5.72 ± 0.18	5.62 ± 0.20
WBC[1] (cm^2)	11.42 ± 1.16	11.02 ± 0.97	9.77 ± 1.12
Cooking loss (%)	21.98^{b} ± 1.12	21.82^{b} ± 0.47	18.81^{a} ± 0.90
Pigment content (ppm)	74.26^{a} ± 3.27	78.09^{a} ± 2.52	93.10^{b} ± 4.41
Shear force (kg)	12.09^{a} ± 1.23	16.18^{b} ± 1.05	18.15^{c} ± 1.88

[1] Water binding capacity;
[a, b, c] Means within rows bearing different letters are significantly different at $p<0.05$.

Conclusion

Results of the investigation indicate that increase of pre-slaughter weight has impact on decrease of dressing percentage and chill loss and increase of share of fat tissue in kid carcass. Heavier kids had higher share of first category parts (leg and loin) and also a better values of meat quality parameters, such as: more intramuscular fat, higher water binding capacity and lower cooking loss. Based on results presented in this study it can be concluded that by slaughtering of heavier

kids (at 22 kg of live weight), meat of better quality is obtained without detrimental impact on carcass characteristics.

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Karakteristike trupa i neki parametri kvaliteta mesa jaradi balkanske rase koza zaklanih pri različitim masama

M. Žujović, N. Stanišić, Z. Tomić, N. Maksimović, Z. Bjelić, V. Mandić

Rezime

U radu je ispitivan uticaj mase pred klanje na karakteristike i sastav trupa i kvalitet mesa jaradi. Ogled je izveden na 18 jaradi balkanske rase koza, zaklanih sa težinom od: 10, 15 i 22 kg. Teža jarad su imala manji randman toplog i hladnog trupa u odnosu na lakša i veći udeo masnog tkiva u trupu. Gubitak mase pri hlađenju bio je najmanji (4.00%) kod jaradi težine 22 kg, što je verovatno posledica veće debljine potkožnog masnog tkiva. Povećanje mase pred klanje jaradi utiče na povećanje udela intramuskularne masti u uzorcima *M. longissimus dorsi*, koji se kretao od 1.51% za jarad težine od 10 kg do 2.09% za jarad težine od 22 kg ($p<0.05$). Tehnološki kvalitet mesa je bio bolji kod težih jaradi, koja su imla veću sposobnost vezivanja vode i manji kalo kuvanja. Rezultati prikazani u ovom ogledu potvrđuju pozitivan efekat povećanja težine pred klanje na karakteristike trupa i kvalitet mesa.

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RESEARCHES REGARDING THE INDUSTRIAL CROSS EFFECT BETWEEN DIFFERENT BREEDS OF SHEEP OVER THE GROWING INTENSITY AND THE SLAUGHTER INDICATORS

C. Pascal[1], C. Costica[2]

[1]Faculty of Animal Science, University of Agricultural Sciences and Veterinary Medicine Iasi, Romania
[2]Agricultural Station of Research and Development for Sheep and Goat Growing Secuieni-Bacau, Romania
Corresponding author: pascalc@uaiasi.ro
Original scientific paper

Abstract: The purpose of the research was to investigate new solutions for the production of increased sheep meat quantities. The opportunity to carry out these surveys is because Romania has a very good position among the countries with large herds of sheep and that at European Union level requirements for sheep meat are not assured. In order to achieve the objectives set out above we included in the scheme for the production of the biological material for recovery for the main breeds in Romania, namely the Merinos de Palas and Tigaie, at which we add the Bluefaced Leicester and Suffolk. Biological material intended for fattening was a hybrid produced from crossing three-breed females mixed-race F1 (Bluefaced Leiceste x races) with Suffolk rams. Weaning lambs was made at the age of 85 days, and to remove the influence of the age was taken into account that differences in this parameter to be less than 25 days. The technology was applied to fattening of intensive type, and had a total duration of 90 days. Intensity analysis of fattening each batch was weighed at the beginning of each phase, weaning, fattening and started at the end of fattening, and to compare the results, control groups were made. In the case of consignments composed by using the breed of Merinos de Palas, the weight of the control group was lower by 5.2% as compared to the performance by the group composed of hybrid terminals, from which the final live weight was 40 kg. For the following experimental formats by using the Tigaie, the control batch though has the onset of fattening a bodyweight with 8.64 percent compared with the batch composed of three-breed hybrids, final live weight was more than 20% higher in the experimental batch. The results obtained at the fattening, and the quality of the carcasses being much better at experimental batches justifies the practicing of this type of crossing.

Key words: sheep, meat, Tigaie, Romanian breeds, industrial cross.

Introduction

Taking into consideration the agricultural surface, Romania is one of the first countries (situated on the 5th place in Europe, with a share of 8% from the total UE-27). Through the labor force engaged in agriculture and food industry, Romania has all the chances to become a net exporter of food produces, especially the animal once obtained from sheep. Although in Romania the objectives for growing sheep are multiple (milk, meat, wool, skins), lately major efforts are being made to increase meat production. The purpose of these concerns is to make Romania an important provider of sheep meat and to satisfy the request made by the countries situated in the Near East (Lebanon, Iran, Syria, Jordan) for meat originated from the exploitation of young and adult sheep, or those of the countries from the European Union for the meat obtained from the slaughtering of fattened young sheep. To provide breeders more information from this domain, during the researches there were organized studies and experiments starting from hybrid lambs obtained by crossing Romanian sheep with rams from meat breeds, which have the quality to improve this product.

Material and Methods

Biological material used in research was represented by several batches of hybrids produced from crosses with mixed-race mother sheep with rams belonging to breeds with very good skills for the production of meat. In order to assess the skills for the production of meat, the performance made by half breeds were compared with those recorded in the youth batches of maternal breeds, subject to the same conditions of fattening by feeding and maintenance.

Control batches belonged to local breeds Merinos of Palas (M1) and (M2) Tigaie and the experimental batches comprised of three-breed hybrid lambs resulting from the crossing of Suffolk with F1 hybrid females Bluefaced Leicester x Merinos of Palas (L1) and Suffolk with F1 hybrid females Bluefaced Leicester x Tigaie (L2). The weaning of the individuals was carried out at the age of 85 days.

Applied fattening technology was intensive, with a total duration of 90 days and had three phases (accommodation, growth and fattening and finishing). The duration of fattening was administered a ration with optimal structure so that youth can externalize fattening performance in production. To determine the specific daily consumption the feeds were weighed before administration, the remnants remaining unused. At the beginning and end of each phase there were made weighing of the lambs, and based on the results it was determined the total rise, average daily increase and consumption. In statements made to avoid calculation errors induced by the gastrointestinal contents, the concerned individuals were no

longer feed with 12 hours before slaughter. Objective assessment of carcasses was done by determining the following items: table, return to the slaughter, the determination of the physical structure of the carcasses, case classification on quality methods applied in the European Union and cut portions of the case depending on the quality. After the assessments carried out on the carcasses, they were cut into regions for slaughter, and then they were boned to determine the bones/meat ratio, both for housing and for each region cut.

The achieved result have been input into the data base, used to run statistical analysis with the algorithm REML (Restricted Maximum Likelihood), which provide the achievements of the statistical parametric estimators within the normal range.

Results and Discussion

At the end of the period, to assess the performance of fattening, batches have been established. Lambs included in each batch, belonged to the same genotype and in order to avoid the influence of age, the weaning of the lambs was made at 85 days from birth.

The evolution of weight and body mass build-up was assessed by weighing all the individuals at the start and at the end of 90 days of intensive fattening. Analysis of the data shows that the intensity of growth and fattening capacity was different in the four batches set up, which will be presented and analyzed separately, depending on the use of cross breeds of Romania.

In the case of batches obtained using local breed Merinos of Palas, the onset of fattening lambs obtained by using it, the difference in body weight between the batches was favorable to the purebred. These had a higher absolute weight with 2,749 kg, distinct difference significant $P \leq 0.01$. The existence of such situations may be justified only on the basis that the mother sheep of the bred Merinos of Palas had a superior lactose secretion, which allowed the lambs to take full advantage of the specific growth rate from the first postpartum periods. In the specific literature there are reported similar situations which support this claim *(Mireşan et al., 1979; Ionescu et al., 1985; Bekedam et al., 1986; Imangaliera, 1990).*

At the end of the fattening period, the situation observed was different from those found at the onset. The average weight of the consignment of the control, constituted by Merinos of Palas lambs was lower with 5.2% compared with the performance of the batch composed of three-breed hybrid that had at the end of the period a live weight of over 40 kg. In the specific literature there are reported and other similar situations. So, *Inger (1986)* on the basis of the research undertaken shows that half breed Suffolk x Swedish Landrace the body weights were recorded live, at 34.200 kg at the age of 120 days. Also, in another experience *(Pascal,*

1998) shows that the products produced from crosses of the same breed with rams of the local Suffolk breed, the half breed genotype has achieved a higher average body weight with 19,95% compared with the live weight of the purebred Merinos of Palas lambs.

In these circumstances, on the basis of the values recorded at the end of fattening based on the lambs weighing, highlights a reverse situation. Thus, if the average body weight of the onset of the three-breed half breed lambs was reduced with 14.33% they had at the end of fattening a larger body weight with 5.22% compared with the average final weight determined at the purebred lambs. The recovery of the differences from the moment when the batches were made was possible because of the precocity of the half breed, expressed by the higher intensity of growth, and at the time when the process of fattening this batch had a higher weight.

Table 1. Evaluation of accumulations of body mass during fattening and the significance of the differences between the batches using the breed Merinos of Palas (n = 12)

Specification	Merinos of Palas		Suffolk x (BFL x M2)		Statistical significance of differences
	$\overline{X} \pm s_{\overline{x}}$	V%	$\overline{X} \pm s_{\overline{x}}$	V%	
Weight at the beginning of fattening (kg)	19.182 ± 0.843	14.58	16.433 ± 0.289	6.10	(10.19954) F α > 0.01 (8.016627) *
Weight at the end of fattening (kg)	38.227 + 1.020	8.86	40.333 ± 1.045	8.98	(2.065197) < F α 0,05 (4.324789) ns
Increase total (kg)	19.045 + 0.763	13.30	23.900 ± 1.006	14.58	(14.36032) F α > 0.01 (8.016627) *
The average daily growth (g)	210.7 + 8.526	13.30	265,6 ± 11.180	14.58	(14.88003) F α > 0.001 (14.58648) ***

Note: ns-not significant; *-significant; **- distinct significantly; ***- very significant.

In these circumstances, both the increase and the average daily total increase achieved during the fattening period had higher values at the three-breed half breeds. In the case of this batch, due to the fact that the total increase during fattening was higher by about 20.31% and the average daily increase with 20.67% we can conclude that the skills for the production of meat are better expressed at three-breed half breeds.

By analyzing the data obtained at the end of fattening, you may find that the industrial crossbreeding with rams from meat breeds, one of their characteristics,

also noted by other authors *(Mann et al., 1984; Abboud*, 1989; *Imangaliera, 1990; Murat, 1995, 1997; Ionescu et al., 2001; Dawson, 2004; Vicovan et al., 2010)*, namely those of weights at early age (precocity), is very well laid out to the half breed batch. In terms of the variability coefficient, the average values are less than 15% can be said to have been somewhat homogeneous batches (Table 1).

In the case of the Tigaie breed the formed batches were made up of youth of purebred sheep and lambs which were obtained by crossing of hybrid F1 (BL x Ti) and which later were used for breeding with Suffolk breed rams, in order to obtain three-breed hybrids specialized for meat production.

Reported to the data presented in Table 2, we note that in this case the consignment consisting of lambs belonging to local Tigaie breed had at weaning and at the fattening debut a superior bodyweight with 8.64% compared with the batch composed of three-breed hybrids.

Just as in the case of the Merinos of Palas breed, this situation can be justified through two distinct and different points of view:

- a better lactose capacity of the maternal breed, which is justified by the fact that at the Tigaie breed, one of the main conditions in selection is milk production;
- the manifestation of different features of growth and body development in youth categories.

This last statement is supported by the fact that research has found that at the batch of individuals belonging to the local breed the speed of growth was more intense during lactation, and at the batches formed from hybrids, the corporal development records a more increase rate after weaning, this meaning the fattening period.

In these circumstances, as a result of the growth of total accumulated throughout the fattening, we find that at the end of the fattening the three-breed hybrid batch had higher average values with 37,87% compared to the data recorded in the control batch. Statistical processing of the data show that the difference between batches, recorded at the end of the period, had a different degree of significance for the 1% thresholds, and that 5% one.

Table 2. Evaluation of accumulations of body mass during fattening and the significance of the differences between the batches using the race

Consignments	N	Weight at the fattening debut (kg)	Weight at the end of fattening (kg)	Total increase (kg)	The average daily growth (g)	
		$\overline{X} \pm s_{\overline{x}}$	$\overline{X} \pm s_{\overline{x}}$	$\overline{X} \pm s_{\overline{x}}$	$\overline{X} \pm s_{\overline{x}}$	
Tigaie	12	18.785 ± 0.705	32.521 ± 0.511	13.736 + 0,254	152.621 ± 0.350	
Suffolk x (BL x Ti)	12	17.130 + 0.467	39.242 ± 0.258	22.112 ± 0,562	245.68 + 0.213	
Tukey Test					d	s.d.
Weight at the beginning of fattening Tigaie and Suffolk x (BL x Ti)					+ 1.65	*
Weight at the end of fattening Suffolk x (BL x Ti) and Tigaie					+ 4.721	**
$W_{5\%}$ = 1.75; $W_{1\%}$ = 3,67. Note: * significantly; ** significantly different						

The determined values for the average daily increase highlights the outstanding performance achieved by the batch composed of terminal hybrids. At this batch, the rate of accumulation of daily body weight was higher with 37.87% than that of the control batch consisting of youth Tigaie purebred. In both cases it is clear that in expressing the rhythm more intense growth, found at the three-breed hybrids, had an important role and influence of the two breeds of meat used in the crossing. These breeds, that induce to the mixed-race batches better features prove that are very good ameliorative of all indicators of which depends on the production of meat from sheep.

Related to this aspect in an experiment carried out in the year 1968 Kincaid, quoted by *Mochnacs et al. (1978)*, using alternating at two females bathes belonging to different breeds, with two rams as well belonging to different race, but one with a higher weight (Hampshire) and one with a lower weight (Southdown), established between products obtained by using at mating those two rams, an average of 0.476 kg statistically significant. This means that the ram has a direct effect over the weight at birth and consequently for the crossing this effect must be taken into account.

Analyses of the degree of precocity for speed of growth for the youth. The existence of these differentiations between the experimental and control batches may be attributed to the different degree of precocity, a feature broadcast on the two breeds of meat namely Blue Faced and Suffolk. The pace of body development for the two periods of lactation and fattening is highlighted by the values from the figure 1.

The study of the values obtained for the batches at the formation of which the breed Merinos of Palas was used indicates the fact that while the control batch carries out during the period of lactation 19.85% from the final weight, the batch formed of three-breed hybrid individuals only carried out 16.43%. However, the same analysis carried out on the basis of accumulation of body mass during all

periods of fattening highlights the fact that while the total increase for the half breed batch is 59,24% from the final weight, at the control batch the muscle masses collections share the same interval was only 50.88%. Carrying out the same analyses and appraisals for the obtained values from the formed batches of Tigaie lambs and three-breed hybrids Suffolk x (BL x Ti) shows different situations. The batch formed by the Tigaie lambs had a more intense growth increase during the lactation period, accumulating on this range about 57.75% and during the fattening period the accumulation was superior with 42.24%.

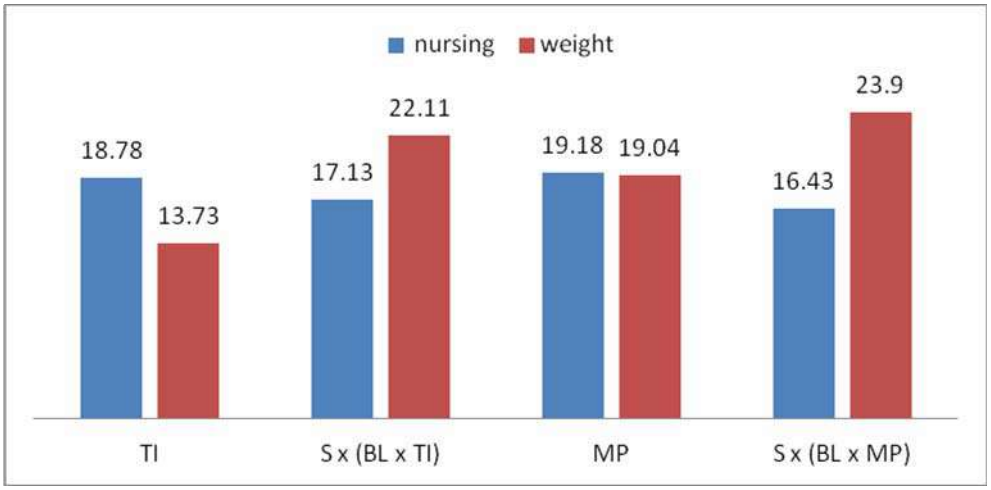

Figure 1. Dynamic accumulations of body weight during lactation and fattening (kg)

The half breed S x (BL x Ti) had at the end an average weight of 39.242 ± 0.258 kg, from which 43.65% was acquired during the lactation period and 56.35% during the fattening. The presence of these differentiations, even in circumstances where an attempt was made to limit the influence of foreign factors indicates that the practice of this type of mating makes it easy to obtain a half breed youth sheep with very good qualities and a very good rate of growth at fattening.

The slaughter yield is an extremely important indicator in the appreciations that aim the meat production. It is influenced by a number of factors, of which nutrition is the most important technology of fattening. In order to limit their influence in research the alimentation was uniform as applied the fattening technology. With regard to this last aspect, several authors cite lower values of slaughter yield in the case of fattening the youth sheep based on the volume forage *(Harington, 1990; Haris, 1990; Borys and Janicki, 2001)*.

At the end of the fattening period, control slaughters were made in order to determine the main quantitative and qualitative properties of the carcasses and meat from the carcasses, as well as the efficiency of the slaughter. In this sense, from each experimental batch, at the end were slaughtered 6 individuals belonging equally to both sexes. In the research conducted on the batches of half breed and purebred lambs, the calculated efficiency was the slaughterhouse and the commercial. The obtained data for the analyzed batches are centralized in table 3.

On the basis of live weight recorded before slaughter and carcasses weighed hot and cold, there were determine average values specific to the calculated yield immediately after slaughter, or at warm and at 24 hours refrigerated (cold).

Table 3. Carcass weight and yield at slaughtering

Specification	Statistics	Genotype			
		Merinos of Palas	S x (BL x Ti)	Tigaie	S x (BL x Ti)
Carcasses weight at hot (kg)	$\overline{X} \pm s_{\overline{x}}$	17.133 ± 0.674	20.833 ± 2.308	14.189 ± 0.331	20.523 ± 0.203
	V%	3.6	5.51	7.2	6.7
	Minimum	16.5	19.0	13.0	19.5
	Maximum	17.5	21.8	14.6	21.0
Carcasses weight at cold (kg)	$\overline{X} \pm s_{\overline{x}}$	16.563 ± 0.658	20.088 ± 1.122	13.789 ± 0.501	19.715 ± 0.355
	V%	6.8	9.7	8.8	6.7
	Minimum	15.5	18.8	12.7	19.5
	Maximum	provides	20.5	13.9	21.0
Hot efficiency (%)	$\overline{X} \pm s_{\overline{x}}$	44.819 ± 0.386	51.652 ± 1.792	43.632 ± 1.417	52.200 ± 0.429
	V%	3.6	6.51	7.2	6.1
	Minimum	43.7	52.2	41.9	49.8
	Maximum	46.5	58.1	43.7	53.5
Cold efficiency (%)	$\overline{X} \pm s_{\overline{x}}$	43.328 ± 0.890	49.805 ± 1.350	42.400 ± 0.231	50.239 ± 0.231
	V%	3.9	4.7	6.5	6.3
	Minimum	43.8	47.5	41. 9	49.8
	Maximum	44. 75	50.0	42.7	50.7

The warm efficiency with the highest values, was recorded in the case of slaughter of the lambs of the half breed batches S x (BL x Ti) and Suffolk x (BL x MP) and have been of 52.20% and 51.65%. The determination of the same indicator for the control batches indicates the existence of some net differences, and in conditions in which the highest value of 44,18% was from the Merinos of Palas and 43.62% from Tigaie it can be concluded that local breeds have much lower average yields than the half breed justifying the point of view of the research. The net differences found were distinctly significant for $p > 5$.

So, using the crossings rams from meat breeds with ewes of indigenous breeds, they have contributed in particular to the increasing of the corporal weight recorded at the end of the fattening period and the weight increases of the carcasses and the slaughter efficiency for half breed lambs.

Conclusion

At the onset of the fattening the weight differences were favorable to both batches composed of lambs local breeds, and the explanation that can be given to this fact would be that the mother sheep of the Merinos of Palas and Tigaie breeds have had a lactation secretion, which allowed the lambs to take full advantage of the growing rate specific to the first postpartum periods.

In the case of the analysis of the influence of the Merinos of Palas breed is apparent that if the average body weight of the onset of the three-breed half breed lambs was lower with 14.33%. At the end of the fattening they had a body mass superior with 5.22% compared with the average final weight determined at the purebred lambs, recovering differences recorded at the time when the batches were formed.

At the end of fattening the three-breed hybrid batch S x (BL x Ti) had higher average values with 37,87% comparative with the recorded data from the control batch, recovering the difference from the forming of the batches.

The statistical processing of the data show that the difference between batches recorded at the end of the periods had a different degree of significance for the 1% thresholds, and that 5%.

The existence of these differentiations indicates a higher degree of precocity and a more accelerated rate after weaning in regards to corporal development at both batches of three-breed hybrids.

Compared with the control batches where the determined efficiency at hot had values below 45% in the case of half breed batches S x (BL x Ti) and Texel x (BL x MP) this indicator presents the average values of 52.20% and 51.65%.

Istraživanja uticaja industrijskog ukrštanja različitih rasa ovaca na intenzitet porasta i klanične pokazatelje

C. Pascal, C. Costica

Rezime

Cilj istraživanja bio je da ispitaju nova rešenja za proizvodnju povećanih količina ovčijeg mesa. Prilika da se sprovedu ova istraživanja je važna jer Rumunija ima veoma dobru poziciju među zemljama sa velikim stadima ovaca i to da na nivou Evropske Unije zahtevi za ovčijim mesom nisu sigurni. U cilju postizanja gore postavljenih ciljeva uključili smo se u šemu za proizvodnju biološkog materijala za oporavak za glavne rase u Rumuniji, odnosno Merinos de Palas i cigaja rase, kao i Bluefaced Lester i Safolk. Biološki materijal namenjen za tov je hibrid nastao trorasnim ukrštanjem sa ženkama mešovitog F1 (Bluefaced Leiceste x rase) sa Suffolk ovnovima. Odvbijanje jagnjadi je napravljen u uzrastu od 85 dana, i da bi se eliminisao uticaj starosti, vodilo se računa da razlike u ovom parametru bidu manje od 25 dana. Tehnologija je primenjena za tov intenzivnog tipa, i u trajanju od 90 dana. Intenzitet analize tova je bio sledeći: svaka grupa životinja je izmerena na početku svake faze, odbijanja, tova na početku i na kraju tova, a rezultate su upoređeni sa kontrolnim grupama. Kod ukrštanja sa rasom merino de palas, težine grla iz kontrolne grupe bila je manji za 5,2% u odnosu na grupu sa terminalnim melezima, kod kojih je konačna telsna masa grla bila 40 kg. Za sledeći eksperiment sa rasom cigaja, kontrolna grupa iako je na početku tova imala veće telesne težine za 8.64 % u poređenju sa grupom trorasnih hibrida, konačna telesna masa je za više od 20% veća u eksperimentalnoj grupi. Rezultati dobijeni u tovu, i kvalitet polutki su mnogo bolji u eksperimentalnim grupama što opravdava korišćenje ove vrste ukrštanja

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SOME NON-GENETIC FACTORS INFLUENCING EARLY GROWTH TRAITS OF LAMBS

V. Caro Petrovic[1], M. P. Petrovic[1], Z. Ilic[2], M. M.Petrovic[1], A. Teneva[3] J. Stojkovic[2], D.Ruzic Muslic[1]

[1]Institute for Animal Husbandry, Belgrade-Zemun
[2]Faculty for Agriculture, Lesak
[3]University of Forestry, 1756 Sofia, Bulgaria
coressponding author: violycaro@yahoo.com
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Abstract: The objective of this study was to investigate the importance of possible contributing non-genetic sources of variation on early growth traits of lambs. The body weight at birth influenced by many genetic and non-genetic factors. Today, among other things, scientists analyzed of genetic biodiversity of some genes related to growth and development of lambs. Genetic improvement through selection in a breeding program confide in the preciseness of identifying genetically premium animals. This requires that non-genetic factors (fixed effects) influencing the accuracy of predicted breeding values be either controlled experimentally or eliminated statistically. The results showed that more of the analyzed factors had a significant effect on body weight at birth and weaning. The effect of year on BWB and BW60 were very significant ($P<0.01$) while at BW30 and BW90 were not significant ($P>0.05$). The effect of sex was very significant at BWB ($P<0.01$) while at the rest ages; BW30, BW60 and BW90 were not significant ($P>0.05$). Birth type and age of dam had significant ($P<0.05$) effect on BWB and very significant ($P<0.01$) at BW30, BW60 and BW90.

Key words: sheep, lamb growth, non genetic factors

Introduction

Growth potential of the lambs is one of the most important traits in a genetic improvement scheme for meat sheep. A number of non-genetic factors affect these growth traits and directly obscure recognition of the genetic potential. Adjustment of data for non-genetic factors and estimation of genetic parameters for the various traits are necessary to obtain reliable estimates for important economic

traits and to increase the accuracy of selection of breeding animals (*Gbangboche et al.,2006;Hussain et al 2013;Caro Petrovic et al.,2012)*.

The genetic improvement through selection in a breeding program confide in the preciseness of identifying the genetically premium animals. This requires that non-genetic factors (fixed effects) influencing the accuracy of predicted breeding values must be either controlled experimentally or eliminated statistically (*Van Wyk et al., 1993*). Prior to the estimation of breeding values, a model must to be specified to found which best describe the biological processes wherein this requires study on non-genetic sources of variation that influence the specific measured trait concerned. The effects of non-genetic factors such as age, sex, type of birth and the age of dam are documented. Early growth traits in sheep are known to be influenced by direct and maternal genetic effects as well as by environmental effects (*Lewis and Beatson, 1999*). From the mother's perspective, maternal effects on progeny performance result from maternal traits controlled by her genotype and associated environmental factors (*Lavvaf et al., 2007*). Therefore, these effects are divided into genetic and environmental components. However, from the side of the offspring, maternal effects are reflected as environmental (*Szwaczkowski et al., 2006*). Wherefore, it is essential for genetic assay, selection programs, animal breeding strategy, breeding value estimation and mating strategy that estimates this components and correcting data for this effects. The body weights and growth rates in pre-weaning are often considered as an early indicator of the late growth and economic benefit (*Hanford et al.,(2006*). In general, growth traits, in particular until weaning are not only influenced by the genes of the individual for growth and environment under which it is raised but also by the maternal genetic composition and environment provided by the dam (*Lewis and Beatson, 1999*).

The objective of this study was to investigate the importance of possible contributing non-genetic sources of variation on early growth traits of lambs. This information is necessary if adjustments for effective selection on genotype are to be made.

Material and Methods

In the investigation conducted was used MIS breed of sheep. The research covered a period of three years. The traits included in the study were: body weight at birth (BW), Body weight at 30 days (BW30), body weight at 60 days (BW60) and body weight at 90 days (BW90). Lambs were measured at birth, with 30, 60 and 90 days of age. All determinations of weight were carried out to the nearest 0.1 kg. During the whole period of study applied the same technology for growing sheep and lambs. After lambing, all lambs were reared in a standard manner till the end of the study period. Feeding lambs was carried out in addition sucking milk,

alfalfa hay and concentrate (*ad libitum*). The dams were grouped into three groups on the basis of their age at lambing: young (< 4 years), mature (from 4.1 to 6 years) and old (> 6.1 years).

The data (100 lambs per classes of effects) were analyzed to estimate the effect of year, sex, birth type and age of the dam on lambs' weight from birth to weaning.

Statistical analysis of data for the understudied traits was done using the SPSS software and the following model.

$Yijklm = \mu + Gi + Sj + Bk + Al + \varepsilon ijklm,$
where:

$Yijklm$ = birth weight of m^{th} lamb of l^{th} age of dam, k^{th} birth type in j^{th} sex and i^{th} year
μ = overall population mean
Gi= effect of year (fixed effect –3 classes)
Jj = effect of sex (fixed effect –2 classes)
Bk = birth type (fixed effect –2 classes)
Al = effect of age of the dam (fixed effect –3 classes)
$\varepsilon ijklm$ = residual error

Results and Discussion

As referred in table 1, the lambs born in year 2 had the highest average birth weight. The difference of weight with the lambs born in year 1 was 0.55 kg (55grams) and with year 3 was 0.28 kg. The lambs born in year 3 had a higher average birth weight than the lambs born in year 1 with a difference of 0.27 kg.

The heaviest were lambs in year 1 compared with in year 2 and 3 for the BW30 and BW90 (Table 1 and 2). The differences were 0.03 kg and 0.11 kg at 30 days, wherein at 90 days differences were 0.07 kg and 1.19 kg. The difference between year 2 and 3 were 0.08 kg for BW30 and 1.12 for BW90.

Table 1. Means and standard errors of body weight from birth to 30 days, kg

Effect		BWB		BW30	
		Mean	SE	Mean	SE
Year	1	3.97	±0.10	11.94	±0.19
	2	4.52	±0.08	11.91	±0.17
	3	4.24	±0.10	11.83	±0.20
Sex	Male	4.55	±0.08	11.93	±0.15
	Female	3.93	±0.07	11.86	±0.15
Birth type	Single	4.38	±0.07	13.19	±0.15
	Multiple	4.10	±0.08	10.60	±0.15
Age of dam	Young	4.23	±0.09	11.90	±0.19
	Mature	4.42	±0.08	13.01	±0.16
	Old	4.08	±0.07	10.77	±0.15

At 60 days (table 2), the lambs body weight were heaviest in year 3 for 0.23 kg in comparison with year 1 and 1.49 kg comparing with year 2. The difference between year 1 and 2 was 1.26 kg.

Males were dominant in body weight in all ages, started from BWB to BW90. The variations of body weight were 0.61 kg at birth, 0.07 kg at 30 days, 0.50 kg at 60 days, and 0.58 kg at 90 days.

As shown in both tables (1 and 2), the lambs born single had a heavier body weight from birth to 90 days of age compared to the lambs born twins.

The lambs born by mature dams had the heaviest body weight compared with lambs of young dam and old dam. The differences of body weight in all ages were the following; 0,19 kg and 0,34 kg (BWB), 1.11 kg and 2.24 kg (BW30), 3.13 kg and 5.02 kg (BW60), 2.55 kg and 5.41 kg (BW90). Lambs of young dam were heavier than lambs' born by old dam. The differences were 0.15 kg (BWB), 1.13 kg (BW30), 1.89 kg (BW60), and 2.86 kg (BW90).

Based on table 3, it showed that the effect of year on body weight of lambs at BWB and BW60 were very significant ($P<0.01$) while at BW30 and BW90 were not significant ($P>0.05$).

The effect of sex was very significant at BWB ($P<0.01$) while at the rest ages; BW30, BW60 and BW90 were not significant ($P>0.05$).

The results in table 3 also showed that birth type and age of dam had significant ($P<0.05$) effect on BWB and very significant ($P<0.01$) at BW30, BW60 and BW90.

Table 2. Means and standard errors of body weight from 60 to 90 days, kg

Effect		BW60		BW90	
		Mean	SE	Mean	SE
Year	1	21.78	±0.30	30.77	±0.42
	2	20.52	±0.26	30.70	±0.36
	3	22.01	±0.30	29.58	±0.42
Sex	Male	21.69	±0.23	30.64	±0.34
	Female	21.19	±0.24	30.06	±0.32
Birth type	Single	24.03	±0.23	33.09	±0.32
	Multiple	18.85	±0.24	27.61	±0.33
Age of dam	Young	21.13	±0.29	30.43	±0.40
	Mature	24.26	±0.24	32.98	±0.34
	Old	19.24	±0.22	27.57	±0.32

Our findings can be compared with results of other researchers. Birth weight of lambs was influenced by various non-genetic factors -period of birth, season of birth, sex of the lamb and parity (*Thiruvenkadan et al 2008).* Lambing year had a highly significant effect on the pre-weaning traits **(***Roshanfekr et al 2009***)**. According to *Petrovic et al.(2009*) the values of the birth weight in lambs of Mis breed, observed by years ranged from 4.43 kg to 4.58 kg.

The birth weight of single born lambs was heavier than twins (table1). Many authors *(Dixit et al., 2001; Mishra et al., 2007; Thiruvenkadan et al., 2009; Abbas et al,. 2010; Albial et al., 2010. Gamasaee et al., 2010)* stated that the effect of birth type was significant on birth weight of lambs and it can be explained by limited uterine space and nutrition of lamb during pregnancy. *Petrovic et al.(2009)* stated that type of birth has also expressed a significant effect on the body mass of Mis lambs at birth. Variations ranged in the interval from 4.31 kg (twins) to 4.59 kg (single).

According to *Caro Petrovic et al.(2012*), in Lipska and Svrljig breed, birth type had significant effect on the body weight from birth to weaning and the highest body weights were recorded in singles. Sex of lambs had a significant effect at birth and very significant effect at 30 days, but had no significant effect on weight at 60 (BW60) and 90 (BW90) days.

Likewise, *Snyman, (1995)* noted, that the year, sex, birth type as well as age of the dam were the important sources of variation for the body weight at all ages, which is true in the result we obtained.

These significant differences in the growth rate of lambs and in different periods maybe attributed to alterations in management, feeding and environmental condition such as the ambient temperature, humidity, rainfall and other factors, which either affects the lambs directly or indirectly through their effects on dams (*Mandal et al., 2003, Thiruvenkadan et al.,2009, Abbas et al.,2010, Petrovic et al., 2011).*

Table 3. Effect of fixed factors on early growth traits of lambs

Effect		BWB	BW30	BW60	BW90
Year	F	8.757	.0760	8.329	2.544
	Sig.	.000	.927	.000	.082
Sex	F	30.280	.115	2.208	1.513
	Sig.	.000	.735	.139	.221
Birth type	F	6.696	139.083	237.656	135.254
	Sig.	.011	.000	.000	.000
Age of dam	F	4.681	52.176	113.064	66.508
	Sig.	.011	.000	.000	.000

Our results were consistent with other authors that the sex of the lambs had highly significant effect on birth weight (*Thiruvenkadan et al., 2008)* effect of sex on birth weight was also reported by *Sivakumar et al. (2006); Ravimurugan et al. (2007).*

Single lambs and male lambs were heavier than twins and females as a stated by *Gbangboche et al. (2006***).** According to *Hussain et al.(2013)* sex had non-significant effect on 90 days weight, which is in compliance with the result we obtained. *Zhang et al. (2008)* reported that the growth advantage of single born at birth might result from less competition for nutrition supply from the dam in gestation period than the multiple birth ones.

The age of dam acts primarily by the variation of the mother dairy production according to the number of lactations *(Najari, 2005). Ouni et al.(2010)*, reported that the age of dam showed no effect on the weights at birth and at the age of 30 days, which was in contrary with our result (table 3). However, the significant effect of dam age on early growth traits have been published with many scientists such as *Snyman et al. (1995), Yazdi et al. (1998) and Esmaeili Zade et al. (2002)* and shown that this effect in different of ages is a curve status and between 4 to 6 years of age is a optimal condition, which is in accordance with our results.

Year of birth, sex, type of birth had significant effects on birth weight and weaning weight *(Abegaz et al., 2002)* this statement concur in our study.

Conclusion

Based on the data, it can be concluded that year, sex, birth type and age of dam had a significant effect on BWB. The birth type and age of dam had significant effect on early growth traits of lambs. The results showed that more of the analyzed factors had a significant effect on body weight at birth and weaning. Therefore, these factors should be included in the operational model suited for the estimation of genetic parameters or expectation of breeding values for the Mis sheep breed. Negligence to do so could result in the restraint of selection progress attributable the use of inaccurate genetic parameters and breeding values in the selection program.

Uticaj nekih negenetskih faktora na porast jagnjadi

V.Caro Petrovic, M. P. Petrovic, Z. Ilic, M. M.Petrovic, A. Teneva J. Stojkovic, D.Ruzic Muslic

Rezime

Cilj ovog rada je bio da se ispita značaj mogućih doprinosa ne-genetskih izvora varijacija na osobine ranog porasta jagnjadi.Telesna masa na rođenju je pod uticajem mnogih genetskih i ne-genetskih faktora. Danas su, između ostalog, naučnici analizirali genetski polimorfizam nekih gena koji se odnose na rast i razvoj jagnjadi. Genetsko unapređenje kroz selekciju u programima oplemenjivanja oslanja se na preciznost identifikacije genetskih performansi životinje. To zahteva da genetski faktori (fiksni efekti) koji utiču na vrednosti fenotipova, budu kontrolisani ili eliminisani statistički. Rezultati ovih istraživanja su pokazali da većina analiziranih faktora imaju značajan uticaj na telesnu masu na rođenju i odbijanju. Uticaj godine na BWB i BW60 je bio veoma značajan ($P<0,01$), dok na BW30 i BW90 nije bio značajan ($P> 0,05$). Uticaj pola bio je veoma značajan kod BWB ($P <0,01$), dok razlike pod uticajem ovog faktora za BW30, BW60 i BW90 nisu bile značajne ($P> 0,05$). Tip rođenja i i starost majke su imali značajan uticaj ($P<0,05$) na BWB i veoma značajan efekat ($P <0,01$), na BW30 BW60 i BW90.

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APPLICATION OF THE NON-HORMONAL METHODS FOR SYNCHRONIZATION OF ESTRUS IN EWES

Ts. Hristova, S. Stoycheva

Institute of Mountain Stockbreeding and Agriculture, Troyan,5600, Bulgaria
Corresponding author: cvetomira_16@abv.bg, s_vetla@abv.bg
Review paper

Abstract: Non-hormonal methods are based on exogenous factors that activate the ovarian function of ewes. They are harmless, economic profitable and easy applicable. They increase the reproductive parameters in farms.
Reproductive efficiency of sheep flocks is the product of three factors: fertility, prolificacy and the lambs' survival. The realization of the resulting offspring in current market conditions provide 95-98% of the income in meat sheep (*Todorov, 2008*), 60% in dairy sheep (*Stancheva and Staykova, 2009*) and 80% in fine-fleece sheep breeds (*Boykovski et al. 2009*). The increase of fertility and year-round production of milk and meat are the main reason for the review made by us.

Key words*:* Sheep, non-hormonal methods, synchronization oestrus, estral season, ancestral.

Introduction

Non-hormonal methods are alternative to the conventional methods of estrus synchronization, an important issue in today's consumer – driven climate (*Martin et al., 2004*). The most popular non-pharmacological methods are: the ram effect, flushing, salt-free-salt diet, the uses of vitamins and mineral supplements (*Tyankov et al., 2000*).

In sheep breeding the separate implementation of non-hormonal methods for oestrus synchronization during anoestral season is rarely practiced. Non-hormonal methods largely succeed in bringing about synchronized oestrus in the reproductive season with early maturing sheep breeds and are also a precondition for improved results from hormonal methods.

The differences in the endocrine function of ewes with different anoestrus duration can influence the fertility at a non-breeding season, particularly in ewes with low fertility. Among the different breed types of ewes used for early lambing

(at a non-breeding season) the best results were obtained from ewes with high fertility (*Jennings, 1973*).

Furthermore the higher-fertile ewes often demonstrate higher capacity to maintain cyclic activity after induced oestrus, i.e. if they are not fertilized during the fist insemination, a great percentage of them are fertilized during the second one (*Gordon, 1996*).

The environmental factors and biologically active substances are used in the non-hormonal methods (*Bankov et al.,1989*).

Stimulation of sexual function for improving the conception rate by feed supplementation is known in practice as the "flushing method". Reproduction results are improved if salt-free diet is implemented for 7-day period, followed by salt-containing one for another 7 days with 15 – 20 g salt per ewe per day, which stimulates the reproductive system via responses.

To flush, producers supplement the breeding flock with good quality hay, fresh pasture or grain for two weeks before and two to four weeks after breeding. During flushing, producers should focus on increasing the amount of energy fed to ewes. In addition to increasing the number of follicles released, elevated energy is believed to increase the number of embryos that implant in the uterus. Boosting these rates increases lambing rates by 10-20%; this is important because a flock's lambing rate is one of the primary factors influencing its economic viability (Kerr, 2006).

Staykova et al. (*2010*) investigated the effect of use non-hormonal methods for stimulation and estrus synchronization in 423 sheep from the Caucasian breed in different body condition. The authors combined schemes of additional nutrition and the ram effect. They found that the best results were obtained by using ram stimulation and applying acute additional nutrition.

The introduction of ram or rams to isolated anoestrus ewes leads to the next reproductive reactions: increase of pulsatile secretion of LH, which may end with LH surge followed by ovulation (*Ungerfeld et al., 2004*). The ram effect is applicable in breeding season too, as the introduction of rams to cyclic ewes stimulates an increase in pulsatile LH secretion, independent of ewe genotype or stage of the estrous cycle (*Hawken et al., 2007*).

The availability of male brood animals accelerates ovulation, which, according to a number of authors, commences 40 – 45 hrs following the contact with rams and is preceded by an ovulation LH-peak. This ovulation remains invisible, that a hidden oestrus is under way that is not accompanied by clinical manifestations. The succeeding oestrus exhibits clinical manifestation and is combined with ovulation (*Oldham and Cognie, 1980; Nugent et al., 1988*).

Metodiev et all. (*2009*) was studied the effect of combined treatment of the salt free-salt diet and the ram effect on main reproductive traits of ewes in different

ages from the Synthetic Population Bulgarian Milk at the beginning of breeding season.

High percent (94.45%) of the experimental ewes reacted positively to the scheme for synchronization of estrus and fertilizations. 89.70% of the ewes were manifested estrus in the framework of 20 days as it was observed 2 surges – between day 1 and day 9 and between day 15 and day 20, after the beginning of the breeding campaign. These results could be explained as a result of the ram effect and salt-free-salt diet and their synergic effect. The authors reported fertility 77.00% and fecundity 127.27%.

The effect of the ram has been proven in many experiments with different breeds showing that it is not similar in different breeds and conditions. Most of the experiments were conducted in Australia and New Zealand, with mainly merino sheep, which is known to have extended breeding season, and they are relatively easy to induce estrus in anestral period (*Ungerfeld et al., 2004*).

Nedelkov et all. (*2011*) have studied the ram effect in ewes from West Balkan Mountain sheep breed. Approximately 58% of the fitted for breeding ewes came in estrous with the characteristic two peaks, within 13 days from 16 to 28 days after the contact with rams, after being isolated from them. The second peak of ewes in estrus is larger than the first in the investigated West Balkan Mountain ewes. By applying the ram effect it was possible to reach over 90% of the ewes to lamb within 36 days, and 40% of them to lamb in the first two weeks.

Among the biologically active substances the vitamins A, D and E have the greatest effect on the reproductive functions. They can be applied by injecting or through the food and water.

The scheme of twofold injecting of trivitaminol (AD_3E) every 7 days at the dose of 5 $см^3$ to the ewes and 10 $см^3$ to the rams in combination with feeding of germinated barley and a salt-free and salt diet was applied in our experiment. The adequate feeding in combination with supplemental feeding with germinated barley (1/3 of the daily ration of concentrated feed) and a salt-free and salt diet conducted 15 days before the beginning of the mating campaign of the ewes, are an effective means of provoking and synchronizing the estrus at the anestrous season in more than 2/3 of the ewes of milk type and sale of lamb meat and ewe's milk at higher prices, by 30 – 35 % and 20 – 25 %, respectively (*Yankov et al., 2004, 2005, 2006*).

A major reserve for increasing the fertility in sheep breeding is the parturition compaction. The parturitions are considered compact when the interval between them is shortened and there is more than one parturition within a year. To achieve this it is necessary 80-90% of sheep in the flock to be mated within 3 to 4 weeks (21-28 days) (*Metodiev et al., 2009*). A great number of scientific and applied experiments carried out by *Bankov et al.* (*1989*) proved that twice-yearly

lambing is difficult to realize because the interval between parturition and fertilization should not be greater than 60 days.

Conclusion

1. The combination of non-hormonal methods is possible and desirable, because this leads to increase the percent of the ewes in estrous, fertility and fecundity.

2. For the conditions of Bulgaria it is recommended to fertilize the ewes in May to the first 10-day period of June and sell lambs to be slaughtered for the Christmas holidays when the market prices are higher (30 – 35 %). In the winter and spring months the ewe's milk is also bought at higher prices (20 – 25 %).

Primena ne-hormonskih metoda za sinhronizaciju estrusa ovaca

Ts. Hristova, S. Stoycheva

Rezime

Ne-hormonske metode su zasnovane na egzogenim faktora koji aktiviraju funkciju jajnika ovaca. Oni su bezopasni, ekonomski isplativi i lako primenljiv. Oni povećavaju reproduktivne parametre na farmama.

Reproduktivna efikasnost stada ovaca je proizvod sledećih faktora: plodnost i opstanka jagnjadi.

Realizacija dobijenog potomstva u trenutnim tržišnim uslovima daju 95-98% prihoda u ovčetine (*Todorov, 2008*), 60% u mlečnom ovčarstvu (*Stancheva i Staikova, 2009*) i 80% kod fino-runih rasa ovaca (*Boikovski i sar. 2009*).

Povećanje plodnosti i tokom cele proizvodnje mleka i mesa su glavni razlog za ovaj pregledni rad.

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FATTY ACID COMPOSITION OF DIFFERENT ADIPOSE DEPOTS IN LAMBS REARED INDOORS AND ON PASTURE

T. Popova*

Institute of Animal Science, 2232 Kostinbrod, Bulgaria
*Corresponding author : tlpopova@yahoo.com
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Abstract: Fatty acid composition of adipose depots was studied in ram lambs of Northeastern Bulgarian Fine Wool breed and cross of this breed with Ile de France, reared indoors and on pasture. Four depots were examined – intermuscular, subcutaneous, perirenal and omental fat. Rearing affected significantly ($P<0.001$) the content of the linolenic acid in the intermuscular, perirenal and omental adipose tissue in the lambs, as well as the content of CLA in the intermuscular ($P<0.001$) and perirenal ($P<0.01$) fat which were higher in the pastured lambs. Oleic fatty acid, the total amount of monounsaturated fatty acids as well as the stearoyl-CoA desaturase activity in the perirenal fat were significantly higher in the lambs reared on pasture ($P<0.05$). The effect of breed of the lambs was most pronounced in the subcutaneous fat as the lambs of the cross had lower amounts of linoleic and linolenic acids ($P<0.05$). Breed significantly influenced ($P<0.01$) the content of oleic acid, total monounsaturated fatty acids and the activity of stearoyl-CoA desaturase which were higher in the crossbred lambs. Lower elongase activity ($P<0.05$) was observed in the omental fat of the lambs of the cross.

Key words: lambs, adipose tissue, fatty acids, rearing, breed

Introduction

The deposition of adipose tissue in the animals is a process of continual accretion of lipids, mainly in the form of triacylglycerols. It is a very important factor that affects the quality of lamb carcasses and meat through both the amount of the adipose deposits and their composition. As one of the main constituents of meat, adipose tissue is responsible not only for the sensory parameters but it is a concentrated source of energy, bioactive substances (vitamins A, D, E and K) and

important fatty acids. Fatty acid profile could be modified using various feeding regimes and rearing strategies although in lambs and other ruminants nutritional factor have less influence due to the biohydrogenation occurring in the rumen (*Wood and Enser 1997, Nurnberg et al. 1998*). In addition to nutrition, other important factors that modify the lipid profile of fat (intramuscular fat and other depot fats such as subcutaneous or renal fat), are breed or breed origin of sheep, their sex and age (*Wood et al. 2008*). Breed of the lambs determines the rate and amount of fat deposited in muscle tissue and other parts of the body. Depending on the location of adipose tissue in the carcass, fat may differ in fatty acid composition and response to the modification of methods used.

The aim of this study is to determine the influence of rearing and breed specifics on the fatty acid composition of various fat depots in lambs.

Material and methods

Experimental animals and feed regimens

The experiment was carried out with 28 male lambs of Northeastern Bulgarian Fine Wool Breed (NBFWB) and lambs crosses of this breed with Ile de France (♀NBFWB x ♂IDF) in the Institute of Animal Science – Kostinbrod. According to their breed the animals were divided in two groups (each containing 14 lambs) and each of these groups was subsequently divided in 2 subgroups (of 7 animals) – one reared indoors and the other reared on pasture. At the beginning of the experiment the mean age and live weight of the animals were 95 (±5) days and 19,47kg (±0.5). Before the onset of the experiment two groups of lambs of NBFWB and the cross received concentrate for 10 days. Hay and water were *ad libitum*. The other two groups received hay which was gradually replaced by fresh grass and the lambs were adapted to pasture. During the experiment the lambs received 620g/d per animal and 420g/d per animal concentrate, respectively for the groups reared indoors and on pasture. The diet consisted of maize 29.5 %, wheat -36 %, sunflower meal -32 %, vitamin premix -0.5 %, lime -2%. The fatty acid profile of the diet is presented on Table 1. The experiment continued 73 d until the animals reached the following live weight - NBFWB: indoors–31.13 kg, pasture -31.80 kg; NBFWB x IDF: indoor - 34.25 kg, pasture-32.32 kg.

Table. 1 Fatty acid composition of the diet

	Concentrate	Hay	Grass
Fatty acids, %			
C14:0	0.20	4.16	1.38
C16:0	11.10	31.82	18.30
C16:1	0.30	1.50	1.18
C18:0	3.60	4.89	3.70
C18:1	26.00	10.09	6.12
C18:2	57.80	20.98	23.21
C18:3	1.00	26.56	46.11

Table 2. Effect of rearing system and breed on the fatty acid composition of subcutaneous adipose tissue in lambs (values least square means)

Fatty acids, %	Rearing system		Breed			Significance		
	Indoors	Pasture	NBFWB	NBFWB x IDF	S.E[1]	Rearing	Breed	Interaction
C14:0	4.13	3.9	4.1	3.93	0.43	NS	NS	NS
C16:0	28.78	27.96	28.11	28.63	1.25	NS	NS	NS
C16:1	1.34	1.39	1.46	1.26	0.36	NS	NS	*
C18:0	20.91	22.42	22.79	20.55	3.03	NS	NS	NS
C18:1	37.48	37.06	35.33[a]	39.21[b]	2.06	NS	**	NS
C18:2	5.66	5.26	6.11[a]	4.81[b]	1.2	NS	*	NS
C18:3	0.47	0.6	0.65[a]	0.42[b]	0.22	NS	*	NS
CLA[2]	1.2	1.36	1.41	1.15	0.26	NS	NS	NS
SFA[3]	53.83	54.3	55	53.12	2.33	NS	NS	NS
MUFA[4]	38.82	38.46	36.8[a]	40.48[b]	2.23	NS	**	NS
UFA[5]	46.16	45.69	44.99	46.87	2.33	NS	NS	NS
AI[6]	0.98	0.95	0.99	0.94	0.05	NS	NS	NS
UFA/SFA	0.86	0.84	0.82	0.88	0.08	NS	NS	NS
SDI[7]	0.44	0.43	0.42[a]	0.45[b]	0.02	NS	**	NS
EI[8]	0.73	0.8	0.81	0.72	0.12	NS	NS	NS

Values connected with different superscripts are significantly different (P<0.05)
Significance effects : *P<0.05; ** P<0.01; NS- non significant
[1]S.E- standard error
[2]CLA- conjugated linoleic acid
[3]SFA – total saturated fatty acids = Σ C14:0+C16:0+C18:0
[4]MUFA-total monounsaturated fatty acids = Σ C16:1+ C18:1
[5]UFA- unsaturated fatty acids = ΣC16:1+C18:1+C18:2+ C18:3 +CLA
[6]AI- atherogenic index = ((4xC14:0)+C16:0))/(C16:1+C18:1+C18:2+C18:3+ CLA)
[7]SDI- Stearoyl-CoA desaturase index= (C16:1+C18:1)/(C16:0+C16:1+C18:0+C18:1)
[8] EI- Elongase index = C18:0/C16:0

Slaughtering and sampling

At the end of experiment 5 animals of each group were slaughtered. Samples for fatty acid composition of the perirenal and omental fat were taken immediately after slaughter while those of the intermuscular and subcutaneous fat were taken after storing the carcasses at 4 º C for 24 h.

Fatty acids analysis of the adipose tissue

Total lipids of the four depots were extracted according to the method of *Bligh and Dyer (1959).* Methyl esters of triacylglycerols, isolated by preparative TLC were obtained using 0.01 % solution of sulphuric acid in dry methanol for 14 h, as described by *Christie (1973)*. The fatty acid composition of triacylglycerols was determined by GLC analysis using chromatograph C Si 200 equipped with capillary column (TR-FAME - 60 m x 0.25 mm x 0.25μm) and hydrogen as a carrier gas. The oven temperature was first set at 160 º C for 0.2 min, then raised until 220 º C at a rate of 5ºC/min and hold for 5 minutes. The temperatures of the detector and injector were 200 º C. Methyl esters are identified comparing to the retention times of the standards. Fatty acids are presented as percentages of the total amount of the methyl esters *(Christie 1973).*

Statistical analysis

Data was analyzed using two way analysis of variance (ANOVA). The mathematical model included fixed effects ascribed to rearing system (indoors and pasture), breed (NBFWB and NBFWB x IDF) and rearing x breed interaction on the fatty acid composition of the adipose depots in the lambs. Whenever necessary, the means were compared through the Student t-test. Differences with a level of significance below 0.05 were considered significant. Statistical analyses were performed using JMP, version 7 software.

Results and discussion

Influence of the rearing system on the fatty acid composition of the adipose depots

The effect of the rearing system (pasture vs. indoors) was observed in the intermuscular, omental and perirenal adipose tissue, concerning the individual

polyunsaturated fatty acids of these fat depots. The rearing influenced significantly (P<0.001) the content of linolenic acid C18:3 in the intermuscular adipose tissue (Table 3). The same was observed in both omental and perirenal adipose tissue (Tables 4 and 5). Our results are consistent with those of studies of *Nurnberg et al. (2001, 2005)*. The higher percentage of the linolenic acid in the pastured lambs is due to the considerate quantities of this fatty acid in the grass (Table 1) and shows that despite the processes of the rumen certain amounts of polyunsaturated fatty acids escape biohydrogenation. According to *Ben Salem et al.(1993),* the process of biohydrogenation could be diminished if the grass in not fed in crushed or ground form.

Table 3. Effect of rearing system and breed on the fatty acid composition of intermuscular adipose tissue in lambs (values least square means)

Fatty acids, %	Rearing system		Breed		Significance			
	Indoors	Pasture	NBFWB	NBFWB x IDF	S.E.[1]	Rearing	Breed	Interaction
C14:0	4.52	4.2	4.82	3.91	1.19	NS	NS	**
C16:0	24.02	22.78	23.61	23.19	2.58	NS	NS	NS
C16:1	1.01	0.89	0.9	1	0.28	NS	NS	NS
C18:0	26.13	27.6	27.28	26.44	3.66	NS	NS	NS
C18:1	34.97	35.36	33.93[a]	36.41[b]	2.39	NS	*	*
C18:2	7.73	6.7	7.37	7.05	1.52	NS	NS	NS
C18:3	0.49[a]	0.9[b]	0.67	0.72	0.12	***	NS	NS
CLA[2]	1.09[a]	1.53[b]	1.38	1.25	0.24	***	NS	NS
SFA[3]	54.59	54.68	55.72	53.54	2.36	NS	NS	*
MUFA[4]	35.99	36.26	34.83[a]	37.41[b]	2.45	NS	*	*
UFA[5]	45.31	45.4	44.27	46.45	2.36	NS	NS	*
AI[6]	0.94	0.87	0.97	0.84	0.17	NS	NS	*
UFA/SFA	0.83	0.83	0.79[a]	0.89[b]	0.07	NS	*	*
SDI[7]	0.41	0.41	0.40	0.43	0.02	NS	NS	NS
EI[8]	1.1	1.23	1.19	1.15	0.27	NS	NS	NS

Values connected with different superscripts are significantly different (P<0.05)
Significance effects: *P<0.05; ** P<0.01; ***P<0.001; NS- non significant
[1]S.E- standard error
[2]CLA- conjugated linoleic acid
[3]SFA – total saturated fatty acids = Σ C14:0+C16:0+C18:0
[4]MUFA-total monounsaturated fatty acids = Σ C16:1+ C18:1
[5]UFA- unsaturated fatty acids = ΣC16:1+C18:1+C18:2+ C18:3 +CLA
[6]AI- atherogenic index = ((4xC14:0)+C16:0))/(C16:1+C18:1+C18:2+C18:3+ CLA)
[7]SDI- Stearoyl-CoA desaturase index = (C16:1+C18:1)/(C16:0+C16:1+C18:0+C18:1)
[8] EI- Elongase index = C18:0/C16:0

Table 4. Effect of rearing system and breed on the fatty acid composition of omental adipose tissue in lambs (values least square means)

Fatty acids, %	Rearing system		Breed			Significance		
	Indoors	Pasture	NBFWB	NBFWB x IDF	S.E.[1]	Rearing	Breed	Interaction
C14:0	3.44	3.34	3.35	3.44	1.1	NS	NS	NS
C16:0	22.96	23.65	22.85	23.76	3.17	NS	NS	NS
C16:1	0.81	0.7	0.67	0.84	0.29	NS	NS	NS
C18:0	29.12	31.44	31.53	29.03	4.93	NS	NS	NS
C18:1	33.03	30.67	31.08	32.62	3.6	NS	NS	NS
C18:2	8.65	7.58	8.22	8	2.11	NS	NS	NS
C18:3	0.53[a]	0.9[b]	0.69	0.73	0.16	***	NS	NS
CLA[2]	1.41	1.69	1.56	1.54	0.28	NS	NS	NS
SFA[3]	55.53	58.45	57.74	56.24	3.33	NS	NS	NS
MUFA[4]	33.85	31.37	31.76	33.46	3.76	NS	NS	NS
UFA[5]	44.46	41.54	42.25	43.75	3.33	NS	NS	NS
AI[6]	0.83	0.89	0.86	0.86	0.15	NS	NS	NS
UFA/SFA	0.8	0.71	0.73	0.78	0.1	NS	NS	NS
SDI[7]	0.39	0.36	0.36	0.38	0.04	NS	NS	NS
EI[8]	1.29	1.13	1.41[a]	1.01[b]	0.32	NS	**	NS

Values connected with different superscripts are significantly different (P<0.05)
Significance effects: *P<0.05; ** P<0.01; NS- non significant
[1]S.E- standard error
[2]CLA- conjugated linoleic acid
[3]SFA – total saturated fatty acids = Σ C14:0+C16:0+C18:0
[4]MUFA-total monounsaturated fatty acids = Σ C16:1+ C18:1
[5]UFA- unsaturated fatty acids = ΣC16:1+C18:1+C18:2+ C18:3 +CLA
[6]AI – atherogenic index = ((4xC14:0)+C16:0))/(C16:1+C18:1+C18:2+C18:3+ CLA)
[7]SDI- Stearoyl-CoA desaturase index = (C16:1+C18:1)/(C16:0+C16:1+C18:0+C18:1)
[8] EI – elongase index = C18:0/C16:0

Rearing system affected significantly the content of conjugated linoleic acids (CLA) in the intermuscular fat (P<0.001) and perirenal fat (P<0.01) (Tables 2 and 5). It was higher in the pastured lambs and corresponded to the increased content of linolenic fatty acid in these fat depots. This is in agreement with the results *Guler and Atkumsek (2011)* for lambs raised on pasture. *French et al. (2000)* found that pasture rearing enhanced the content of CLA in ruminal fat and *Kaszor et al. (2010)* observed 15.2 % higher content of CLA in pastured lambs,

compared to lambs fed forages. In many studies so far CLA has been clearly shown to be beneficial for the health *(Collomb et al. 2006)* and mainly human intervention studies were evaluated. According to *Tricon and Yaqoob (2006)* some of the effects of CLA may be isomer-specific.

Table 5. Effect of rearing system and breed on the fatty acid composition of perirenal adipose tissue in lambs (values least square means)

Fatty acids, %	Rearing system		Breed			Significance		
	Indoors	Pasture	NBFWB	NBFWB x IDF	S.E.[1]	Rearing	Breed	Interaction
C14:0	2.76	2.89	2.9	2.76	1.12	NS	NS	NS
C16:0	20.68	21.04	20.74	20.98	3.17	NS	NS	NS
C16:1	0.68	0.57	0.57	0.68	0.29	NS	NS	NS
C18:0	32.55	35.1	34.01	33.63	4.24	NS	NS	NS
C18:1	32.74[a]	29.74[b]	30.6	31.89	2.49	*	NS	NS
C18:2	8.82	8.25	9.03	8.03	2	NS	NS	NS
C18:3	0.52[a]	0.91[b]	0.74	0.69	0.14	***	NS	NS
CLA[2]	1.21[a]	1.47[b]	1.37	1.31	0.19	**	NS	NS
SFA[3]	55.53[a]	58.45[b]	57.74	56.24	2.49	*	NS	NS
MUFA[4]	33.43[a]	30.32[b]	31.17	32.58	2.52	*	NS	NS
UFA[5]	43.99[a]	40.96[b]	42.33	42.62	2.49	*	NS	NS
AI[6]	0.72	0.8	0.77	0.75	0.2	NS	NS	NS
UFA/SFA	0.78[a]	0.69[b]	0.73	0.74	0.07	*	NS	NS
SDI[7]	0.39[a]	0.36[b]	0.36	0.38	0.03	**	NS	NS
EI[8]	1.59	1.73	1.7	1.62	0.38	NS	NS	NS

Values connected with different superscripts are significantly different (P<0.05)
Significance effects: *P<0.05; ** P<0.01; ***P<0.001; NS- non significant
[1]S.E- standard error
[2]CLA- conjugated linoleic acid
[3]SFA – total saturated fatty acids = Σ C14:0+C16:0+C18:0
[4]MUFA-total monounsaturated fatty acids = Σ C16:1+ C18:1
[5]UFA- unsaturated fatty acids = ΣC16:1+C18:1+C18:2+ C18:3 +CLA
[6]AI-atehrogenic index= ((4xC14:0)+C16:0))/(C16:1+C18:1+C18:2+C18:3+ CLA)
[7]SDI- Stearoyl- CoA desaturase index =(C16:1+C18:1)/(C16:0+C16:1+C18:0+C18:1)
[8] EI – elongase index =C18:0/C16:0

According to *Beriain et al. (2003)*, the adipose tissue in lambs contains more than a hundred fatty acids and the most abundant are palmitic C16:0, stearic C18:0 and oleic C18:1. In this study we did not observe effect of the way of rearing

on the amount of palmitic and stearic acids in the four depots. However in another experiment with lambs we found that the amounts of myristic and palmitic acid were at average two times higher in lambs reared on pasture *(Banskalieva et al. 2005).* The results of this study are similar to those of *Nurnberg et al.(2008)*, who did not observe changes in the amount of palmitic and stearic fatty acid in the adipose tissue of lambs but are in contrast to those of *Guler and Atkumsek (2011)*. According to the latter, the amount of the palmitic, stearic and myristic acids in omental and perirenal fat of pasture-fed lambs were higher than those of concentrate-fed lambs.

Rearing system influenced significantly ($P<0.05$) the content of oleic acid in the perirenal adipose tissue (Table 5). It appeared to be lower in the lambs reared on pasture. Two possible explanations for the changes in the content of the oleic acid exist. Since it could be derived from the feed one possibility is that the diet of the pastured animals contained much less of the oleic acid (Table 1). The main site for fatty acid synthesis **de novo** in lambs is the adipose tissue as the main product is palmitic acid. It can be further elongated to stearic acid and desaturated to oleic by the action of the stearoyl-CoA desaturase. According to *Daniel et al. (2004)* pasture feeding in lambs leads to decreased ratio between the stearoyl – CoA desaturase (SCD) mRNA and the acetyl-CoA carboxylase, that means higher content of oleic acid in the adipose tissue in the pastured lambs. Stearoyl-CoA desaturase activity in this study is described by the desaturase index calculated as a ratio (C16:1+C18:1)/(C16:0+C16:1+C18:0+C18:1), as developed by *Malau Aduli et al.(1998*). It was significantly lower in the perirenal fat of the lambs reared on pasture and corresponded to the significantly lower total amount of the monounsaturated fatty acids. *Taniguchi et al. (2004)* found that the SCD mRNA expression level was related to the amount of MUFA in ruminants and described single nucleotide polymorphism, which contributed to a higher MUFA percentage and hence a lower melting point of the intramuscular fat.

Total saturated fatty acid content was slightly higher in the lambs reared on pasture but the difference was significant only in the perirenal fat ($P<0.05$) where we observed a lower ratio between total unsaturated/saturated fatty acids. Our results are in agreement with those of *Guler and Atkumsek (2011)* and could be explained with the presence of the grass in the diet of the lambs which stimulates and provokes more intensive processes of the biohydrogenation in the rumen, compared to the animals, reared indoors.

Fats that have high atherogenicity index value are assumed to be more detrimental to the human health *(Ulbricht and Southgate 1991)*. In the present study, rearing did not influence the values of the atherogenic index but they were slightly higher than those determined by *Kaszor et al. (2010)* in lambs. This seems to be related to the changes in the content of saturated and monounsaturated fatty

acids that did not reach the level of significance required to be statistically different.

Influence of the breed on the fatty acid composition of the adipose tissue

The differences observed in the lipid profile of the adipose tissue depending on the breed specific of the lambs were more pronounced in the subcutaneous (Table 1) and intermuscular fat (Table 2). Breed affected significantly the content of the oleic acid C18:1 ($P<0.01$) as well as that of the linoleic C18:2 and linolenic C18:3 acids ($P<0.05$) in the subcutaneous adipose tissue. Oleic acid was higher in the crossbred lambs and corresponded to the higher total amount of the monounsaturated fatty acids and the values of the stearoyl-CoA desaturase index. Similarly in the intermuscular fat we observed significant influence ($P<0.05$) of breed specific on the content of oleic and total monounsaturated fatty acids. However, it is difficult to interpret this effect conclusively due to the significant interaction with rearing ($P<0.05$). Our results are in agreement with those of *Kaszor et al. (2010)*, who found that crossing sheep of local dairy breed with rams of Ile de France led to higher monounsaturated fatty acids and stearoyl-CoA desaturase activity in the adipose tissue of the crossbred lambs. The higher amount of monounsaturated fatty acids in the lambs of NBFWB x IDF could probably be due to Ile de France breed which is known to deposit more fat in earlier age *(Maia et al. 2012)* and the difference of fatty acid composition depends on the fatness. This corresponds to the higher amounts of carcass and internal fat in the crossbred lambs of this experiment (unpublished data). Lower values of elongase index were observed in the adipose depots of the crossbred lambs as significant effect of breed was observed in the omental fat ($P<0.01$). This corresponds to the higher stearoyl-CoA desaturase activity found in the cross and confirms that genetic groups differ in the expression of the activity of stearoyl-CoA desaturase and elongase, as reported earlier *(Oka et al. 2002, Pitchford et al. 2002).*

Conclusion

Rearing system (indoors *vs.* pasture) had significant influence on the content of the individual polyunsaturated fatty acids. The contents of linolenic acid in the intermuscular, perirenal and omental adipose tissue as well as that of CLA in the intermuscular and perirenal fat were higher in the lambs reared on pasture. Oleic fatty acid, the total amount of monounsaturated fatty acids and the stearoyl-CoA desaturase activity in the perirenal fat were significantly influenced by the rearing and were higher in the lambs reared on pasture.

Breed effect on the fatty acid composition was more pronounced in the subcutaneous fat of the lambs. Crossing the sheep of NBFWB with Ile de France rams led to higher amount of oleic acid and monounsaturated fatty acids as well as decreased content of linoleic and linolenic fatty acids. Higher values of Stearoyl-CoA index in the subcutaneous fat and lower elongase activity in the omental fat was observed in the lambs of the cross.

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Sastav masnih kiselina različitih depoa masnog tkiva kod jagnjadi koja se gaji u zatvorenom prostoru i na pašnjaku

T. Popova

Rezime

Sastav masnih kiselina različitih depoa masnog tkiva ispitivan je na jagnjadima muškog pola severoistočne bugarske finorune rase i meleza ove rase sa il de frans rasom, odgajanih u zatvorenom prostoru i na pašnjaku. Četiri depoa su ispitivana – intermuskularno, potkožno, bubrežno i masno tkivo maramice. Sistem gajenja uticao je značajno ($P<0,001$) na sadržaj linolenske kiseline u

intermuskularnom, bubrežnom i masnom tkivu maramice jagnjadi, kao i na sadržaj CLA u intermuskularnom (P <0,001) i bubrežnom (P<0,01) masnom tkivu jagnjadi koja su bila više na pašnjacima. Sadržaj oleinske kiseline, ukupan sadržaj mononezasićenih masnih kiselina, kao i aktivnost Sterol-CoA desaturaze u bubrežnom masnom tkivu bile su značajno veće kod jagnjadi uzgajanih na pašnjacima (P<0,05). Uticaj rase jagnjadi je imao najveći uticaj na sastav potkožnog masnog tkiva, jer su jagnjad melezi imala niži sadržaj linolne i linolenske kiseline (P<0,05). Rasa je značajno uticala (P <0,01) na sadržaj oleinske kiseline, ukupnih mononezasićenih masnih kiselina i aktivnost Sterol-CoA desaturaze, koji su bili veći u meleza. Slabija aktivnost elongaze (P<0,05) zabeležena je u masnom tkivu maramice kod jagnjadi meleza.

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CHARACTERISTICS OF SJENIČKI EWE'S CHEESE WITH PROTECTED DESIGNATION OF ORIGIN

A. Popović-Vranješ[1], A. Kasalica[2], F. Bauman[3], M. Jevtić[4], D. Cvetanović[1], G. Grubješić[1]

1 Faculty of Agriculture, Trg D. Obradovića 8, University of Novi Sad, 21000 Novi Sad, Republic of Serbia
2 JPS Dairy Institute, Auto put 3, 11070 Novi Beograd, Republic of Serbia
3 Union of breeders of sheep and goats of Serbia
4 Faculty of Medicine, Hajduk Veljkova 3, Novi Sad, Serbia
Corresponding author: Anka Popović-Vranješ, E-mail anka.popovic@gmail.com
Original scientific paper

Abstract: Product with protected designation of origin, Sjenički cheese, is produced exclusively in the area of the municipality of Sjenica and Tutin and the wider region of Sjenica-Pešter plateau. Sjenički ewe´s cheese is made from milk of indigenous sjenička Pramenka ewe, also named sjeničko-pešter ewe. Sjenica cheese production takes place in rural areas and in the mountains and during the summer, in cabins. Ripening of sjenički cheese from ewe´s milk is based on activity of autochthonal microflora, where lactococci and lactobacilli dominate, and are responsible for special sensory characteristics of cheese, giving it a specific odour, taste and distinctive quality. This paper examines the dominant microflora of lactic acid bacteria (LAB) involved in the ripening process, monitoring the total number of lactobacilli, mesophilic and thermophilic lactococci and enterococci, as well as their isolation. The results of our study show that lactobacilli accounted for dominant microflora during cheese ripening. Total number of lactobacilli ranged from 7.98 log cfu/g at the beginning of ripening to 7.23 log cfu/g after 60 days of ripening, mesophilic lactococci from 7.22-6.79 log cfu/g, thermophilic lactococci 6.76-6.09 log cfu/g and enterococci from 6.41-3.81 log cfu/g. During cheese ripening, a decrease was spotted in the total number of all lactic acid bacteria (LAB). However, despite the overall drop in the number of examined bacteria, they retained a high level at the end of ripening, which is in favor of their biological value. The results for microbiological safety of all the cheeses produced from raw milk, showed that the cheeses were microbiologically safe, because no *Listeria monocytogenes* and *Salmonella* species are found in 25 g cheese samples. The results show that cheese is hygienically safe in regards to coagulase positive staphylococci, considering that all the determined values are $\leq 10^4$ cfu/g.

Keywords: sjenički cheese, sjenička Pramenka, autochthonous microflora

Introduction

The product named "Sjenica ewe's cheese" was introduced into Geographic Trademark Register according to the decision of Intellectual Property Office of the Republic of Serbia from December 2012. That ensured the registration of the geographic origin trademark for the Sjenički ewe's cheese produced only in the area of Sjenica municipality and Tutin and the wider region of Sjenica-Pešter plateau in accordance with the description of the geographic area and enclosed geographic map, and identical to those listed in the Study for trademark protection of "Sjenički ewe's cheese" by characteristics and production process. Part of the study that refers to the physicochemical and microbiological characteristics as well as to the sensory evaluation, and that the registration was based on, will be presented in this paper.

Material and Methods

Larger Sjenički cheese manufacturers from the Sjenica region were surveyed, and the production technology was was monitored in batches during six days. Cheese was examined right after production, then during ripening (after 1 and 2 months of ripening and 4 months of storage). Physicochemical analyses were done according to standard methods *(Carić et al., 2000)*, while sensory evaluation was done by expert commission *(Havranek et al., 2012)*.

From the microbiological aspect, testing was performed for dominant microflora of lactic acid bacteria (LAB), that are a part of the ripening process of Sjenički cheese by monitoring a total number of *Lactobacillus* sp*,* mesophilic and thermophilic *Lactococcus* sp and *Enterococcus* sp, as well as their isolation right after production and after 30 and 60 days of ripening.

For counting and isolation of lactobacilli, a Rogosa agar (Merck) was used, incubated at 37^0C for 72 h in anaerobic conditions, for mesophilic lactococci M17 agar (Merck) was used, incubated at 30^0C for 48 h in aerobic conditions, for thermophilic lactococci M17 agar was used, incubated at 44^0C for 48 h in aerobic conditions, and for enterococci, Kanamycin esculin azide agar (Merck) was used, incubated at 37^0C for 48 h in aerobic conditions. Anaerobic conditions were achieved using Anaerocult A bags (Merck), while conditions were checked with Anaerotest strips (Merck).

Microbiological safety of cheeses made from raw milk, was tested right after the production and after 30 and 60 days of ripening in accordance with the Rulebook on general and specific conditions of food hygiene in all phases of production, processing and circulation *("Official Gazette of the Republic of Serbia", no 72/2010)* considering criteria of safety for *Listeria monocytogenes* (Section 1, point 1.1) and *Salmonella* species (Section 1, point 1.10), as well as hygienic criteria in the manufacturing process for coagulase positive staphylococci (Section 2, point 2.2.3). Referential methods were used for detection and counting of *Listeria monocytogenes* and identification of *Salmonella* species, as well as coagulase positive staphylococci, in accordance with current ISO standards for each bacteria.

Additionally, presence of other bacteria in cheese was also tested: *Escherichia coli* (TBX agar) and *Proteus sp.* (SS agar), HiMedia bases were used for detection of all listed bacteria.

Statistical processing has encompassed determining statistically significant differences (t-test), standard deviations (σ) and variation coefficient (CV) according to methods of *Žižić et al., (2006)*.

Results and Discussion

The cheese composition must be in accordance with the Quality Rulebook *(Official Gazette of the Republic of Serbia, No. 33/2010)*, which means that it must contain ≥45% and <60% fat in solids (fat cheese) and more than 67% water in the fat-free solids (soft cheese) and with other parameters defined by the Safety Elaborate *(Popović-Vranješ, 2011)*, Table 1.

Table 1. Physicochemical properties of the Sjenički ewe's cheese

Quality parameters	Range
Fat	20.5-24.6
Solids	37.65-46.62
Fat in solids	45.43-60.23
Moisture	53.38-62.35
Water in fat-free solids	68.92-75.18
Protein	15.11-19.48
Total N	2.912-3.050
Soluble N	0.297-0.302
pH	4.0-4.93
Sodium Chloride	1.64-3.45
Maturity coefficient	9.9-10.2

First picture is of mature sliced cheese and the second one was taken after an assessment of an expert committee for cheeses that got into the Atlas of West Balkan Cheese *(Havranek et al., 2012),* when Sjenički cheese got a maximum number of points in all categories.

Figure 1. Cheese after ripening (crosscut)

Figure 2. Cheese evaluation

The results of microbiological studies show that lactobacilli make up a significant part of the microflora of cheese in the process of ripening. The total number of lactobacilli was somewhere around 7.98 log cfu/g in the beginning of ripening and 7.23 log cfu/g after 60 days of ripening; mesophilic lactococci 7.22-6.79 log cfu/g, thermophilic lactococci from 6.76-6.09 log cfu/g and enterococci 6.41-3.81 log cfu/g (Graph 1). In the process of ripening the number of all observed lactic acid bacteria (LAB) decreased. When we compare the first and 60th day of ripening, lactobacilli, thermophilic lactococci and enterococci exhibited a significant decrease ($P<0.05$), which was not the case with mesophilic lactococci ($P>0.05$). However, even with the decrease of the total number of observed bacteria, their high level is retained even after the ripening period (Table 2.), which testifies of their biological value. Our results are in concord with the Mannu and Pabba study in 2002, as well as Managia et al. in 2008 which state that in the process of ripening the cheese may exhibit changes in the mikroflora or the ratio of LAB bacteria in different stages of ripening. They also show that the conditions in the cheese, important for the growth of bacteria that form a natural culture, were different (E.g. movement of aw and pH in the cheese, ripening temperature, salt content, etc.) and that could affect the growth in different observed cultures. Natural populations of lactic acid fermentation bacteria directly affect the organoleptic characteristics of indigenous cheeses over the biochemical changes during ripening *(Matutinović et al., 2007)*.

Table 2. Total number of lactobacilli, lactococci and enterococci in Sjenički ewe's cheese during ripening (log cfu/g)

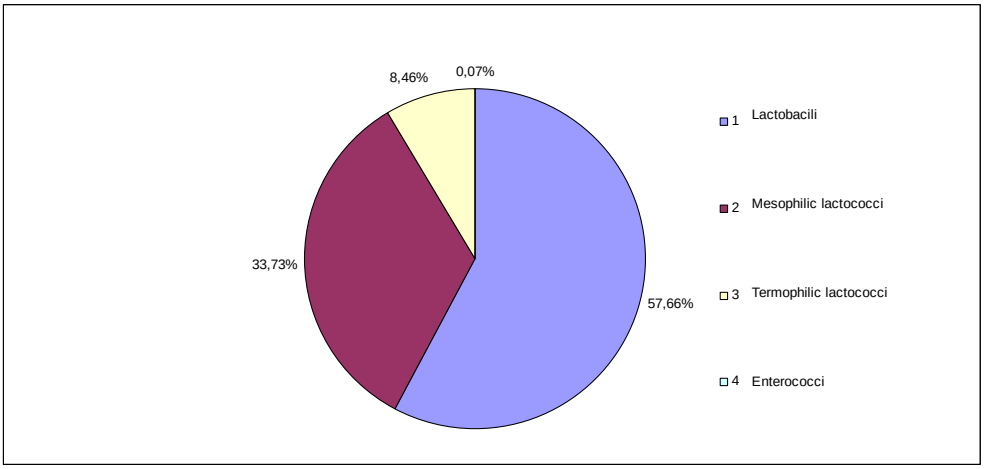
n = 15

Parameter	Days of ripening	Lactobacilli (log cfu/g)	Mesophilic lactococci (log cfu/g)	Thermophilic lactococci (log cfu/g)	Enterococci (log cfu/g)
Average value	1	7.98 [b]	7.22 [NS]	6.76 [b]	6.41 [b]
	30	7.33	6.45	6.73	5.14
	60	7.23 [a]	6.79 [NS]	6.09 [a]	3.81 [a]
Standard deviation	1	0.42	0.72	0.88	0.69
	30	0.42	0.81	0.72	0.82
	60	0.36	0.67	0.70	0.81
Range	1	7.04- 8.28	6.00 – 8.30	5.60 – 7.95	5.43- 7.34
	30	6.81- 7.98	5.30- 7.98	5.82 – 7.95	4.11 – 6.45
	60	6.78 – 7.98	5.74 – 7.66	4.85 – 7.30	2.78 – 5.28
CV	1	5.31	10.01	13.08	10.78
	30	5.76	12.60	10.72	15.97
	60	4.97	9.84	11.45	21.40

[a,b] Statistically significant difference ($P<0.05$)
[NS] Statistically insignificant difference ($P>0.05$)

Percentage of all tested LAB in the cheese after 60 days of maturing have shown that Lactobacilli make up 57.66% of total cheese microflora, when 33.73% mesophilic lactococci were dominant compared to thermophilic lactococci 8.46%, while participation of the enterococcus is at the lowest at 0.07%, as is shown in the Graph 1. This percentage of LAB points that it is a type of autochthonous cheese in whose ripening lactobacilli and lactococci play the most important part; they also give special sensory characteristic to this cheese, when compared to many other cheeses of Southern Balkans where enterococcus make up the dominant microflora at the end of ripening *(Gimenez-Pereira, 2005; Terzić-Vidojević et al., 2007)* and have a significant role in forming sensory properties of those cheeses *(Giraffa, 2003)*.

Graph 1. Percentage of dominant micro flora in Sjenički ewe's cheese after 60 days of ripening (cfu/g)



The presence of different microflora in autochthonous cheeses originates from LAB that enter cheese from raw milk and milking equipment and are involved in ripening of the cheese *(Poznanski et al., 2004)*. The most frequent LABs are from *Lactobacillus, Enterococcus*, *Lactococcus*, *Leuconostoc* and *Pediococcus* genera *(Klein, 2003; Temmerman et al., 2003; Gimenez-Pereira, 2005)*. During our research, 158 types were isolated (*Lactobacillus* spp., *Lactococcus* spp. and *Enterococcus* spp.), that will be the subject of our further research.

Results of microbiological safety of all tested cheeses made out of raw milk, have shown that cheese were microbiologically satisfying considering the criteria of safety, because no *L. monocytogene*s and *Salmonella* species were found in 25 g cheese samples, and satisfying by hygienic criteria in manufacturing process because all determined values for coagulase positive staphylococci were $\leq 10^4$cfu/g and as such are compliant with regulations of the Rulebook *("Official Gazette of the Republic of Serbia", no 72/2010)*. Also, no *E. coli* and *Proteus* sp. were found.

The performed analyses and sensory evaluation of Sjenički cheese show that this is a high quality cheese and highly valuable foodstuff that deserves to be protected.

Conclusion

Research within the Study of physicochemical, microbiological and sensory characteristics of Sjenički ewe's cheese, the main goal, the registration of the geographic origin trademark, was achieved.

Based on the results, it can be concluded that it is the very presence of the autochthonous microflora during the ripening, dominated by lactobacilli and lactococci that affects the specific sensory characteristics of this cheese, giving it a specific odour, taste and distinctive quality.

Protection of Sjenički cheese, at the same time means an encouragement to rural development through introducing different models of production, active involvement of all participants into the distribution chain and preservation of tradition and cheese production for future generations. The importance of protecting autochthonous cheeses is reflected in the protection of consumers, who get a complete information about the product with a guarantee of quality and authenticity.

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Karakteristike sjeničkog ovčijeg sira sa zaštićenim imenom porekla

A. Popović-Vranješ, A. Kasalica, F. Bauman, M. Jevtić, D. Cvetanović, G. Grubješić

Rezime

Proizvod sa imenom porekla Sjenički ovčiji sir proizvodi se isključivo na području opština Sjenica i Tutin, odnosno na širem regionu sjeničko-pešterske visoravni. Sjenički ovčiji sir se proizvodi od mleka autohtone rase ovaca - sjeničke pramenke koju zovu i sjeničko-pešterska ovca. Proizvodnja sjeničkog sira se odvija u seoskim domaćinstvima i na planinama u letnjem periodu u katunima (bačije). Proces zrenja sira zasniva se na aktivnosti autohtone mikroflore, koju dominantno čine laktobacili i laktokoke što utiče na posebne senzorne karakteristike sjeničkog sira, dajući mu specifičan miris, ukus i prepoznatljiv kvalitet. U radu je ispitana

dominantna mikroflora bakterija mlečne kiseline (BMK) koje učestvuju u procesu zrenja, praćenjem ukupnog broja laktobacila, mezofilnih i termofilnih laktokoka i enterokoka, kao i njihovu izolaciju. Rezultati naših ispitivanja pokazuju da su laktobacili činili dominantnu mikrofloru tokom zrenja sireva. Ukupan broj laktobacila se kretao od 7,98 log cfu/g na početku zrenja do 7,23 log cfu/g posle 60 dana zrenja, mezofilnih laktokoka od 7,22-6,79 log cfu/g, termofilnih laktokoka od 6,76-6,09 log cfu/g i enterokoka od 6,41-3,81 log cfu/g. Tokom zrenja sireva došlo je do pada ukupnog broja svih ispitivanih bakterija mlečne kiseline (BMK). Međutim, i pored pada ukupnog broja kod ispitivanih bakterija zadržan je njhov visok nivo na kraju zrenja, što ide u prilog njihovoj biološkoj vrednosti. Rezultati mikrobiološke ispravnosti svih ispitivanih sireva proizvedenih od sirovog mleka, pokazali su da su sirevi bili mikrobiološki zadovoljavajući u pogledu kriterijuma bezbednosti i kriterijuma higijene u procesu proizvodnje prema važećem Pravilniku *("Službeni glasnik RS", broj 72/2010)*.

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THE EFFECT OF SELECTION ON FATTY ACIDS COMPOSITION OF CARCASS FAT IN LORI-BAKHTIARI SHEEP

M. A. Talebi

Department of Animal Science, Research Center of Agriculture and Natural Resources, Shahrekord, Iran.
Corresponding author:maitalebi@yahoo.com
Original scientific paper

Abstract: A study with 56 male lambs of Lori-Bakhtiari sheep breed was carried out to investigate the effect of selection to decrease fat on the fatty acids composition in the subcutaneous fat and fat-tail adipose tissue at constant age. Subcutaneous fat and fat-tail fat samples were obtained from the back of left side of carcass and fat-tail, respectively. Total 112 samples of subcutaneous fat and fat-tail adipose tissue were obtained from 56 carcasses at six month of age. The fatty acid composition of subcutaneous fat and fat-tail were analyzed after fat extraction by gas chromatography. Data were analyzed using SAS statistical program. Proportion of C16:0 in subcutaneous fat and fat-tail significantly decreased and C18:2 significantly increased at the end of the selection program. Ratios of polyunsaturated fatty acids to saturated fatty acids were significantly higher in subcutaneous fat at the end of the selection. Desirable fatty acids and health fatty acids were significantly improved in subcutaneous fat and fat-tail at the end of the selection. Proportion of palmitic and stearic and total saturated fatty acids were significantly higher in subcutaneous fat but oleic fatty acid was significantly higher in fat-tail. Selection for decreased carcass fat and fat-tail also will improve fatty acid composition.

Key words: Selection, Fat-tail, Subcutaneous fat, Fatty acid composition, Lamb.

Introduction

Like most animal production traits, fatty acid composition is influenced by both genetic and environmental factors. The fatty acid composition of fat usually has little influence on market value of the carcass, for which the quantity of fat is of greater importance. However, physical and chemical properties of lipids affect eating and keeping qualities of meat. Meat flavor is influenced by fatty acid

composition *(Melton, 1990)*. Genetics and environment influence meat flavor, species is the most important genetic factor, and feed source is the most important environmental factor *(Shahidi and Rubin, 1986)*.

Ruminant animals in general and sheep in particular, have relatively high ratios of saturated: monosaturated fatty acids in their lipids *(Christie, 1981)*. Lori-Bakhtiari is a fat-tailed breed of sheep, with a population more than 1.6 million. Relative to other Iranian fat-tailed breeds Lori-Bakhtiari is a large breed, having the largest fat-tail. The climatic condition, production systems of nomadic or transhumant pastoralists, poor environmental conditions, economic limitations as well as the requirements of the people, forced the sheep producers in many areas to select for higher fat-tail weight across generations. However, nowadays, producing and marketing of lean lamb to meet the consumer demand for less fat has become a challenge for livestock industry, particularly in countries possessing fat-tail sheep. This is mainly because of the possible association between high levels of dietary saturated animal fats and cardiovascular disease *(Kempster et al. 1986)*. Success in increasing lean tissue deposition while decreasing fat tail, entails breeding from genotype that are genetically superior for lean tissue deposition and inferior for fat-tail deposition. The objective of this study was to compare the fatty acid composition of subcutaneous fat and fat-tail of carcass Lori-Bakhtiari fat-tailed lambs in the selection program for decrease fat at constant age.

Material and Methods

In this study, total 112 samples of subcutaneous fat and fat-tail lambs were obtained from 56 carcasses with average weight 21.93±3.12 kg. The samples of fat had been collected at the first and at the end of the selection program for decrease carcass fat during five years. All of the data came from Lori-Bakhtiari sheep breeding station in Chaharmahal and Bakhtiari province, Iran. The station flock was kept generally from December to May inside the barn and the sheep were fed with alfalfa, barley and wheat stubbles, and they were grazed on range and cereal remainder in other months of the year. The breeding season was from late August to late October and ewes were assigned randomly to the rams. About 15 days after parturition, the lambs were creep-fed during the suckling period. The creep-ration consisted of 50% barley, 10% cotton seed meal, 20% wheat bran, 18% dried sugar beet pulp, 1% bone meal, 0.5% salt and 0.5% vitamin, mineral and antibiotic supplement. The ration was ground, mixed and fed *ad libitum*. Lambs had also access to free choice alfalfa hay.

The lambs were weaned at 90 ± 5 days of age. After weaning, female lambs were kept on pasture and male lambs remained in drylot until six months of age. The ration, fed to male lambs, contained alfalfa hay, barley, beet pulp, cottonseed

meal, salt and mineral supplements, which were ground and mixed.The ration fed ad libitum in post weaning period. The diet contained 14.04% crude protein, 2.50 MCal ME/Kg dry matter and 7.4% Ash. Lambs were slaughtered at six month of age. After slaughtering and skinning, all the abdominal and thoracic organs were removed. The warm carcass was weighed immediately after dressing and removal of offal parts. The carcasses were chilled at 3-2°C for approximately 18 h, and then cold carcasses were weighed and separated into right and left sides. The right side was disjointed into the commercial wholesale cuts traditionally offered in Iran. The method of cutting is described by *Farid (1991)*. Subcutaneous fat and fat-tail fat samples were obtained from the back of left side of carcass and fat-tail, respectively.

The fatty acids composition of subcutaneous fat and fat-tail were analyzed after fat extraction and methylation by gas chromatography. Fatty acids determined were capric (C10:0), lauric (C12:0), myristic (C14:0), palmitic (C16:0), stearic (C18:0), oleic (C18:1), linoleic (C18:2) and linolenic (C18:3). The fatty acids were calculated as the percentage by weight of the total fatty acids observed. Total saturated fatty acids (SFA) was calculated as the sum of C12:0, C14:0, C16:0 and C18:0 while total poly unsaturated fatty acids (PUFA) as the sum of C18:2 and C18:3. Desirable fatty acids (DFA) was calculated as the sum of unsaturated fatty acids and C18:0 but health fatty acids (HFA) as the ratio of (C18:0+C18:1):(C16:0). Data were analyzed by the GLM procedure of *SAS (2005)*. The statistical model for the analyses of fatty acids composition traits included fixed effects due to year of selection (at the first of the selection program and at the end of the selection program), type of fat (subcutaneous fat and fat-tail), type of birth of lamb (single and twin) and age of lamb at slaughter as covariate.

Results and Discussion

The average, standard deviation and coefficients of variation for body weight and cold carcass of the lambs at the first and at the end of the selection program for decrease carcass fat are presented in Table 1. Body weight and cold carcass of the lambs were lighter at the first of selection program than at the end of the selection program. While the coefficients of variation for body weight and cold carcass of the lambs were higher at the first of selection program. Therefore, variation in body weight of the lambs was less with selection. Body weight affected on fat content and fatty acids composition *(Dhanda et al., 2003; Rhee et al., 2003)*.

The effects of the selection year and fat kind and type of birth factors on fatty acid composition at constant age are shown in tables 2 and 3. Proportion of myristic (C14:0), palmitic (C16:0) and total saturated fatty acids were significantly lower at the end of selection program, but proportion of C18:2 and ratios of

monounsaturated fatty acids and poly unsaturated fatty acids to saturated fatty acid were significantly higher. Desirable fatty acids and health fatty acids were improved at the end of selection.

Table 1. Least-square means (±s.e) traits in Lori-Bakhtiari male lambs

Trait	At the first				At the end			
	No	Mean (Kg)	Std (Kg)	CV (%)	No	Mean (Kg)	Std (Kg)	CV (%)
Body weight	27	43.64	4.69	10.75	29	46.68	3.16	6.78
Cold carcass	27	19.99	2.88	14.39	29	23.74	2.13	8.95

Proportion of C10:0 and C14:0 were significantly higher in single born lambs. Saturated fatty acids were no differences in single and twin lambs. DFA were significantly better in twin lambs.

Percentage some of short chain fatty acids (<C16) in subcutaneous fat were similar to their in fat-tail. Proportion of palmitic and stearic and total saturated fatty acids were significantly higher in subcutaneous fat but oleic fatty acid was significantly higher in fat-tail. Ratios of mono unsaturated fatty acids and poly unsaturated fatty acids to saturated fatty acids were significantly higher in fat-tail than in subcutaneous fat. There was a higher degree of saturation in the subcutaneous fat than in the fat-tail. Saturated fatty acids from different investigations vary from 50 to 70% in subcutaneous fat *(Zygoyiannis et al., 1985; Gaili and Ali, 1985; Banskalieva, 1996)*. There were significantly differences in desirable fatty acids and health fatty acids between subcutaneous fat and fat-tail. Desirable fatty acids and health fatty acids were significantly better in fat-tail. Average percentages of DFA were reported between 63.97 and 71.81 in lamb carcass cuts *(Banskalieva et al., 2000)*.

Proportion of C16:0 in subcutaneous fat and fat-tail significantly decreased and C18:2 was significantly increased at the end of the selection program. Ratios of poly unsaturated fatty acids to saturated fatty acids were significantly higher in subcutaneous fat at the end of the selection. In paper of *Banskalieva et al. (2000)*, the PUFA: SFA ratio for different muscles ranged between 0.07 and 0.26 for lamb /mutton, with a median of 0.19. *Sheradin et al. (2003)* reported PUFA: SFA ratio ranged between 0.047 to 0.080 for lamb. Those results are in agreement to our results for proportion PUFA: SFA ratio.

Desirable fatty acids and health fatty acids were significantly improved in subcutaneous fat and fat-tail at the end of the selection program. The fatty acid composition in carcass fat at the end of the selection program was more favourable in respect of health. These results indicate that a genetic approach may be useful to improve fatty acid composition of carcass fat in Lori-Bakhtiari lambs. These results are in agreement with those that *Mezöszentgyörgyi et al. (2001)* reported for study the effect of genotype on the fatty acid composition in sheep.

Table 2. Least-square means (±s.e) of fatty acid composition in carcass Lori-Bakhtiari male lambs at constant age

Fatty acids composition	Effect					
	Year of selection		Type of fat		Type of birth	
	At the first	At the end	Fat-tail	Subcutaneous	Single	Twin
C10:0	0.89±0.06[a]	0.72±0.06[a]	0.90±0.05[a]	0.71±0.05[b]	0.89±0.05[a]	0.72±0.05[b]
C12:0	0.66±0.05[a]	0.66±0.06[a]	0.62±0.04[a]	0.63±0.04[a]	0.68±0.04[a]	0.57±0.05[a]
C14:0	6.14±0.20[a]	5.35±0.20[b]	5.70±0.17[a]	5.79±0.17[a]	6.01±0.16[a]	5.48±0.18[b]
C16:0	31.86±0.37[a]	29.64±0.35[b]	30.11±0.31[a]	31.39±0.31[b]	31.15±0.30[a]	30.34 ±0.33[a]
C18:0	13.94±0.49[a]	14.90±0.47[a]	12.56±0.42[a]	16.28±0.42[b]	13.20±0.41[a]	15.64±0.44[b]
C18:1	40.87±0.72[a]	42.46±0.70[a]	44.21±0.61[a]	39.12±0.61[b]	42.09±0.60[a]	41.24±0.65[a]
C18:2	3.83±0.13[a]	4.70±0.13[b]	4.34±0.11[a]	4.19±0.11[a]	4.17±0.11[a]	4.36±0.12[a]
C18:3	1.73±0.14[a]	1.51±0.13[a]	1.58±0.12[a]	1.66±0.12[a]	1.54±0.12[a]	1.70±0.12[a]
SFA	53.49±0.74[a]	51.21±0.71[b]	49.88±0.62[a]	54.81±0.62[b]	51.94±0.61[a]	52.75± 0.66[a]
MUFA	40.87±0.72[a]	42.46±0.70[a]	44.21±0.61[a]	39.12±0.61[b]	42.09±0.60[a]	41.24±0.65[a]
PUFA	5.56±0.24[a]	6.21±0.24[a]	5.92±0.21[a]	5.85±0.21[a]	5.71±0.20[a]	6.06±0.22[a]
PUFA: SFA	0.11±0.01[a]	0.12±0.01[b]	0.12±0.00[a]	0.11±0.00[b]	0.11±0.00[a]	0.12±0.00[a]
PUMFA:SFA	0.79±0.03[a]	0.84±0.03[a]	0.90±0.03[a]	0.73±0.03[b]	0.84±0.03[a]	0.80±0.03[a]
DFA	54.91±0.56[a]	57.48±0.54[b]	56.88±0.48[a]	55.51±0.48[b]	55.40±0.47[a]	56.99±0.50[b]
HFA	1.74±0.04[a]	1.95±0.04[b]	1.90±0.04[a]	1.79±0.04[b]	1.80±0.04[a]	1.89±0.04[a]

Table 3. Least-square means (±s.e) of fatty acid composition for interaction between type of fat and year of selection in carcass Lori-Bakhtiari male lambs at constant age

Fatty acids composition	Effect			
	Type of fat × Year of selection			
	Fat-tail		Subcutaneous	
	At the first	At the end	At the first	At the end
C10:0	0.96±0.08[a]	0.84±0.08[a]	0.82±0.08[ab]	0.61±0.08[b]
C12:0	0.65±0.07[a]	0.60±0.07[a]	0.67±0.07[a]	0.59±0.07[a]
C14:0	6.05±0.26[ab]	5.35±0.25[b]	6.23±0.26[a]	5.35±0.25[b]
C16:0	30.86±0.48[b]	29.36±0.46[c]	32.85±0.48[a]	29.92±0.46[bc]
C18:0	12.09±0.65[b]	13.03±0.62[b]	15.78±0.65[a]	16.78±0.62[a]
C18:1	43.51±0.96[a]	44.90±0.92[a]	38.23±0.96[b]	40.02±0.92[b]
C18:2	4.03±0.17[b]	4.64±0.17[a]	3.62±0.17[b]	4.76±0.17[a]
C18:3	1.77±0.18[a]	1.39±0.18[a]	1.70±0.18[a]	1.63±0.18[a]
SFA	50.60±0.97[bc]	49.17±0.93[c]	56.38±0.97[a]	53.25±0.93[b]
MUFA	43.51±0.96[a]	44.90±0.92[a]	38.23±0.96[b]	40.02±0.92[b]
PUFA	5.80±0.32[ab]	6.03±0.31[ab]	5.32±0.32[b]	6.38±0.31[a]
PUFA: SFA	0.12±0.01[a]	0.12±0.01[a]	0.09±0.01[b]	0.12±0.01[a]
PUMFA:SFA	0.87±0.04[ab]	0.93±0.04[a]	0.70±0.04[c]	0.77±0.04[bc]
DFA	55.72±0.74[bc]	58.05±0.72[a]	54.10±0.74[c]	56.92±0.72[ab]
HFA	1.81±0.06[b]	1.99±0.05[a]	1.66±0.06[c]	1.92±0.05[ab]

SFA=Saturated Fatty Acids; MUFA=Mono unsaturated Fatty Acids; PUFA=Poly Unsaturated Fatty Acids; PUFA: SFA=Ratio of Poly Unsaturated Fatty Acids to Saturated Fatty Acids; PUMFA:SFA= Ratio of Poly and Mono Unsaturated Fatty Acids to Saturated Fatty Acids; DFA (desirable fatty acids)= all unsaturated fatty acids and C18:0; HFA (health fatty acids)= (C18:0+C18:1):(C16:0).
Means with different superscripts (a–c) are significantly different ($P < 0.05$) in each row for certain effect.

Conclusion

Consequently, according to the present study, the fatty acid composition in carcass fat at the end of the selection program was more favourable in respect of health. Proportion of polyunsaturated fatty acids and saturated fatty acids indicate that a genetic approach can be useful to improve fatty acid composition of carcass fat in Lori-Bakhtiari lambs.

Uticaj selekcije na sastav masnih kiselina masnog tkiva trupa ovaca rase lori-bakhtiari

M. A. Talebi

Rezime

Studija sa 56 jagnjadi Lori-Bakhtiari rase ovaca je sprovedena da se ispita uticaj selekcije na smanjenje masti u sastavu masnih kiselina u potkožnom masnom tkivu i masnog tkiva masti-repa pri konstantnom uzrastu. Uzorci potkožnog masnog tkiva i masti repa su dobijeni iz zadnje leve polutke trupa i masti repa, respektivno. Ukupno 112 uzoraka potkožnog masnog tkiva i masnog tkiva repa uzeto je sa 56 trupova, starosti šest meseci. Masne kiseline iz potkožnog masnog tkiva i masti repa su analizirane nakon ekstrakcije masti gasnom hromatografijom. Podaci su analizirani korišćenjem statističkog programa SAS. Udeo C16: 0 u potkožnom masnom tkivo i masti repa značajno je smanjen i C18: 2 značajno povećao na kraju odgajivačkog programa. Odnosi polinezasićenih masnih kiselina i zasićenih bili su značajno veći u potkožnom masnom tkivu na kraju selekcije. Poželjne masne kiseline i zdrave masne kiseline poboljšane su značajno u potkožnom masnom tkivu i masti repa na kraju selekcije. Udeli palmitinske i stearinske i ukupnih zasićenih masnih kiselina bili su značajno veći u potkožnom masnom tkivu, ali oleinske masne kiseline bio je značajno veći u masti repa. Selekcija na smanjenje masnog tkiva trupa i masti repa takođe će poboljšati sastav masnih kiselina.

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THE POSIBILITY OF THE CERTAIN ALTERNATIVE CEREALS CULTIVATION FOR ANIMAL NUTRITION IN CLIMATIC AND SOIL CONDITIONS OF THE REPUBLIC OF SERBIA

R. Pivić[1], Đ. Glamočlija[2], D. Jošić[1], A. Stanojković-Sebić[1], Ž. Dželetović[3]

[1]Institute of Soil Science, 11000, Belgrade, Republic of Serbia
[2] University of Belgrade, Faculty of Agriculture
[3] Institute for Application of Nuclear energy
Corresponding author: drradmila@pivic.com
Review paper

Abstract: Cereals have a very important role in animal nutrition. Their fruits - grains are used in making concentrated animal feed. Particularly important for animal nutrition, besides barley, oat, maize, sorghum, millet and triticale grain, can be certain alternative cereals such as buckwheat, amaranth and foxtail millet, since climatic and soil conditions of cultivation in the Republic of Serbia correspond to those of the growth and development of these crops. Realized yields may justify the investment in production and also for animal nutrition.

Key words: alternative cereals, buckwheat, amaranth, foxtail millet, climate, soil

Introduction

The large number of plants belongs to the group of cereals. These species are, according to the cultivation and use of the main grain products and by-products (*Glamočlija et al.*, 2012), very similar, although they belong to different botanical families. Most cereals belong to the family of annual and perennial grasses, or fam. Poaceae Barhart. Buckwheat is a member of the family Polygonaceae L., and amaranth - of the family Amaranthaceae.

Bran, that remain in the complex process of the grain grinding, are the main component of the animal feed. In addition, most of the cereals can be grown for green biomass, which is used as animal feed as fresh bulk animal feed, dried (hay) or for making haylage and silage.

For the production of bulky feed, cereals can be sown as pure crops or, more often, in mixtures with grain legumes and oilseed rapes. Further in the paper, a particular attention is paid to the climatic and soil conditions of growing buckwheat, amaranth and foxtail millet in the agricultural areas of the Republic of Serbia.

Climatic conditions in the Republic of Serbia

Most of the territory of the Republic of Serbia is mountainous, and less is flat. In relation to an altitude (*Maksimović et al*., *2008*), in the Republic of Serbia can be conditionally set aside the following rayons:

Lowland region, which includes areas with an altitude up to 300 m. Climatic characteristics of this region are manifested with long, warm and often fervent and dry summers. Winters are shorter, but they sometimes could be quite severe.

Highland (transition) region includes the hilly and hilly-mountainous area. It is located within 300-500 meters above the sea level and it is a particular climatic zone, which lies in the transition between the other two.

Mountainous region extends over 500 m above the sea level and covers mountain massifs. This area has mountainous-continental climate with long and cold winters, and fresh and short summers, as well as with extremely higher precipitation. Snow cover in some places reaches several meters and stays for several months.

The climatic conditions of the territory of the Republic of Serbia are generally favorable, particularly thermal potential, while uneven distribution of rainfall and its seasonal variation causes increased risk, thus causing the need for the implementation of complex amelioration measures (*Pivić*, *2013*).

A part of the Republic of Serbia, which includes the lowlands part, river valleys and gently rolling hill areas, has a mean annual air temperature between 10 and 11^{o}C. The average annual temperature in areas with an altitude up to 300 m for a period of monitoring the climatic parameters in the Republic of Serbia (1981-2010, the source: RHMS of Serbia) is 10.9^{o}C, and in the highland region is about 10.0^{o}C. In mountainous with an altitude above 1000 m mean annual temperatures are 3.6-7.7 ^{o}C (*Glamočlija et al., 2012*).

Autumn is warmer than spring. The coldest month is January, with an average monthly temperature ranging from -4.6 to-2.1^{o}C in the mountainous area; in the highland area the country varies from -0.4 to 1.4 ^{o}C. The warmest month in the Republic of Serbia is July, with values vary from -1.6 to-0.1 ^{o}C, while the temperature interval in the lowland areas of the an average monthly temperature ranging from 11.0 to 22.0^{o}C.

Lowland area has a mean temperature in July that ranges from 21.5 to 23.5°C, while in the highland area the temperature ranges from 20.0 to 21.6°C. Above 1000 m the warmest month is August, when the air temperature is in the range from 12.8 to 17.5°C.

In all locations in Serbia the warmest month is July and the coldest month is January. The minimum annual temperature fluctuations are in mountainous areas, and the largest are on sites with enhanced continental thermal climat.

Regarding the atmospheric processes and relief characteristics, precipitation on the territory of the Republic of Serbia are irregularly distributed in time and space. The average annual sum of precipitation, based on the processed data for the period of observation (1981-2010), for the entire country is 684.1 mm. Annual precipitation sum in average increase with an altitude. The most of the Serbian country has continental rainfall regime, with higher amounts in the warmer part of the year.

The most rain falls in May and June. In June falls 12 to 13% of the total annual rainfall. The least rainy months are February and October, with an average of 5 to 6% of the total annual rainfall. The area of the southwestern Serbia, due to terrain, slopes of the high mountain massifs and the influence of the Mediterranean climate, has a Mediterranean rainfall regime with the maximum in November, December and January, and the minimum in August.

The occurrence of snow cover is characteristic for the period from November to March, and sometimes it occurs in April and October, while on the mountains above 1000 m can also occur during other months. During the period of observation (1981-2010), January is the month with the largest number of days with snow cover, when occurs the average of 30 to 40% of the total annual number of days with snow cover.

The annual sums of solar radiation, registered in the Republic of Serbia, ranged from 1500 to 2200 hours per year. During the warmer part of the year, in Serbia prevail winds from the northwest and west. During the colder part of the year east and southeast wind - Košava prevails. In the mountainous areas in the southwest of Serbia prevail winds from the southwest. For the summer period is important to note the appearance of the southern winds, when many soils are dried, especially combined with high.

Soil conditions

The Republic of Serbia occupies an area of 88.361 km^2. Agricultural areas cover about 64.6%. Agricultural soils, represented in Serbia (*Maksimović et. al*, *2008, 2009*), are in the range of very fertile, without limiting factors in agricultural

production (chernozem, brown forest soil, alluvium) to those with medium and low fertility (smonitsa, pseudogley, semigley).

The pedological cover of the Republic of Serbia is very heterogeneous (Škorić et al.,1985, 1986). The soils of Serbia (*Antonović and Vidaček*, *1979, 1980*) can be classified in eight quality classes, which represent the level of capability of productive soils for use in agriculture. The first four quality classes are soils that are more or less suitable for cultivation. Those are mostly soils located up to 500 m above the sea level, and on a slope of up to 30% mainly deep with favorable agro-ecological properties and not so pronounced seasonal humidification.

According to the degree of the use limitation in intensive agricultural production, the soils suitable for cultivation are divided into four classes. The first has no limitations or they are insignificant, while the fourth requires the protective rotation or the technical measures of protection against erosion, or occasionally prevention of impacts of groundwater or surface water, and as such can be considered conditionally suitable for cultivation. Below in the paper certain characteristics of the soils suitable for the cultivation and growing the crops are presented.

The first quality class includes a very good soils, deep, with loamy texture, permeable, well-drained, with neutral reaction, and suitable for cultivation.

The second quality class includes medium deep soils, sandy, loamy or clayey, permeable to harder permeable, well to moderately drained, with neutral to slightly acid reaction, on flat or gently sloping relief. They are exposed to periodic floods and weak erosion.

To the third quality class belong shallow to moderately deep soils, sandy, loamy, clayey, permeable to harder permeable, with neutral, slightly acid to acid reaction, suitable to mediumsuitable for cultivation.

The fourth quality class includes soils used with serious limitations that narrow the choice of culture to be grow and/or require the special measures of soil protection. These are generally shallow to moderately deep skeletoidal and skeletal, acid soils exposed to flooding. As a measure of using them in intensive agricultural production it is necessary to provide drainage, otherwise the machine work is very difficult.

Alternative cereals for production of animal feed

Below in the paper it will be presented the alternative varietes of cereals that can be grown for the preparation of animal feed in the climatic and soil conditions in the agricultural areas of the Republic of Serbia.

Buckwheat (*Fagopyrum esculentum* Moench) is an annual plant, a crop of spring planting and with a very short growing period (60-90 days). Short growing period allows that the buckwheat can be grown as a follow-up or stubble crop, and in very different environmental and soil conditions.

Regarding the morphological properties, buckwheat is different from the other cereals because it belongs to another botanical family. The root system is spindle-shaped, medium-developed with strong taproot from which a multitude of lateral roots, covered with long root hairs and with strong absorbent power, emerge. They reach a maximum depth of 120 cm. The tree is erect, ribbed, elastic, naked and ankled with 10-15 lateral branches.

The tree height is of 30 to 300 cm. Plants are green with some purple areas that expand with maturing, so the mature crop can be identified by the purple color. The leaf has a simple structure, a petiole (stem) and wide heart-triangular or spear leaf shapes. Over the surface the leaves are smooth, green and soft.

By maturing the plants leaves fall. The flowers are grouped in a complex inflorescence branch, rising from the arm of the side branch. On the plant develop 10-15 branches and total up to 2.000 flowers. The flowers are five-part, white or colored. The flowering is ssuccessive and lasts about 30 days, starting from the base of the tree. Of the total number of flowers about 10% are fertilized, so that on the plant develop 40-50 fully formed fruits. The fruit nut (grain) is triangular, dark colored.

The weight of 1.000 fruits is 25-30 g, and density is 50-70 kg. Peeled fruit has 9% of the total proteins, 70% of starch, 4% of cellulose, 2% of oil, 2% of mineral salts, vitamins B_1 (thiamine), B_2 (riboflavin), B_3 (pantothenic acid) and vitamin E (tocopherol). The fruit has a total of 4.290 mg of indispensable amino acids, which is significantly more than in those of true cereals - wheat and rye.

Smaller grains, which are not suitable for peeling, are used as animal feed because they have a great protein value. Crop residues have a higher protein content, but less digestible carbs than cereals straw. Aboveground biomass can be used for animal feed as fresh, silaged or dried. The highest quality has the biomass of the plants at the end of blooming.

Growing conditions: Buckwheat, as a crop of spring to late summer sowing, grows very well in the relatively wet and cool climate of the mountainous areas because it has a short period of maturity in the technological stage (of two to three months), depending on the usage. The plant does not tolerate temperatures below 10^0C, but also the high thermal values of air. In the terms of soil, it can be grown on poor, acidic and shallow highland soils.

Amaranth (*Amaranthus* sp. L.) is an ancient pseudo-cereal that the peoples of Central America introduced into production before 8.000 years. The

utility value of amaranth is high. The total aboveground biomass can be used in the nutrition of humans and domestic animals. The leaves contain significant amounts of mineral salts and vitamins. Mature fruits of seeds have 15-16% of the total proteins, high content of beneficial fibers, and they are rich in vitamins of the group V.

According to the botanical classification, amaranths belong to the genus *Amaranthus*, which has more than 60 wild species. They often occur as weeds in arable crops, especially on fertile soils. Among such a large number of wild species, nowdays we commonly grow the following three:

- Red (natterer) amaranth (*Amaranthus caudatus* L.)
- Mexican (purple) amaranth (*Amaranthus cruentus* L.)
- Decorative (smooth) amaranth (*Amaranthus hypochondriacus* L.).

According to morphological properties, amaranths are very different, and these differences are due to growing in different climatic and soil conditions. The root system is of spindle type with developed taproot and numerous lateral veins that spread in the surface soil layer. The tree is erect, usually unbranched; in the initial stages of growth it is succulent and herbaceous, while during the maturation of plant it calcifies. Tree height varies and depends on the species, form, and growing conditions. In varieties grown for grain, plants are uniform in height, mostly about 150 cm.

The leaves are with simple structure with long leaf stalks and oval-elongated flakes, sized 4-14 cm x 2-6 cm. Leaf size depends on variety and leaf location on the tree. With the growth of the plants larger leaves to the middle of the tree develop, and then decrease to the smallest ones that appear at the top. The leaves and the whole plant can be more or less colored in red or purple. During the day leaves are partially turning towards the sun, or the light source (heliotropic plants).

The leaves are an important source of nutrients. The fresh leaves are with lower calorific value, but they are rich in vitamins of group V (thiamin, riboflavin, niacin and pyridoxine), and in ascorbic acid, tocopherol and provitamin A. In addition, they are an important source of minerals: potassium, calcium, sodium, magnesium, iron, zinc and phosphorus. In the proteins, which are part of the leaves, prevail thefollowing 17 amino acids: isoleucine, leucine, lysine, methionine, alanine, cysteine, fenilalnin, tyrosine, threonine, valine, arginine, aspartic acid, glutamic acid, glycine, histidine, proline and serine.

The flowers are grouped in spikelike or a whisk broom flowers that grow on the tree top or side branches. The blossoms can be erect or curved, different lengths and colors. The inflorescences contain a large number of tiny bipolar flowers of five-part structure, the green or red crown that after fertilization partially envelops the fetus. Amaranth blooms in June and July, with pollination that is

predominantly autogamous, while the one-seed fruits develop in August. In one bloom up to 5.000 fruits can be formed.

Newer varieties, in favorable environmental conditions and with impementation of an appropriate agricultural technology can provide more than 500 grams of grain per plant. An fruit (seed) is small, lens-like shaped, sized 1-2 mm, with weight of 0.6-0.8 g of 1000 seeds. In the varieties for grain the fruits are whitish to light brown color. In the chemical composition of grain the dominant are food carbohydrates (BEM) (about 65%), on the second place are the total proteins (17%) with a favorable amino acid composition (high content of lysine) and gluten-free, while the oil content is up 15%, and mineral salts about 2%.

Growing conditions: Amaranth is sensitive to frost in the early stages of growth and should be sown later in the spring, or when the soil warms up to 15^0C. It tolerates high temperatures, and for the normal development the plants need a lot of light. Early autumn frosts accelerate the plants maturation. It tolerates the drought, amaranth grows the best in deep, fertile soils of neutral reaction. It should not be sown on poor, acidic and very weedy soils.

Foxtail millet - Italian millet (*Panicum miliaceum* ssp. *italicum*) originates from East Asia and according to the archaeological findings it was cultivated in China over 8.000 years ago.

It finds the implementation in human, domestic and farmed animals nutrition, which indicates the high economic importance of this plant species. By-products in industrial processing are the raw materials for further industrial processing or they are added to the concentrated feed.

An unpeeled grain is used for production of the concentrated fodder, ideal for poultry or pigs nutrition, while the vegetative biomass, mostly in the form of hay, is an excellent fodder for horses and ruminants. An unpeeled grain of foxtail millet, in other words of spikelike panicles, are used for feeding the cage birds. The straw, that remains after the harvest of spikelike panicles, can be used as bedding or fodder.

Foxtail millet has great agrotechnical significance. It is characterized by short vegetation period and high genetic fertility potential. In lowland areas it can be grown as a stubble or following crop. It is tolerant to drought and grows in more arid regions of our country without irrigation. Its vertical distribution of growing is great and it can be sown in the mountainous areas up to 1.000 meters above the sea level. Foxtail millet is a good preceding crop because the soil after the harvest is not weedy and has good physical properties.

Foxtail millet is an annual plant and the crop of spring planting. According to the morphological characteristics, it is similar to millet-like cereals. In the germination stage it forms a primary root, while its secondary root system is

strongly developed, deep and with high absortption power. It develops upright and ankled tree with height of 2 m. Tillering intensity depends on the density of planting, variety and used agrotechnical practice.

On the tree, 8-14 leaves of a simple texture develop, with a long, gray-green hairy flakes. The flowers are grouped in a complex inflorescence, meaning, the spikelike panicle of cylindrical, spindle or head shape. In the inflorescence it is formed 3.000-20.000 chaffed fruits. By the shape the fruit is usually oval-elongated, with an absolute mass of 2.0-4.5 g, and density of 72-76 kg.

The share of chaff in the total fruit weight is 20-25%. The fruit has a high nutritional value because it has a 12.5% of total proteins, 53-55% of starch, 1.5% of glucose soluble in water, 11% of cellulose, 4.5% of oil, 2.2% of mineral salts and 13% of water. In the fruits there are vitamins B_1, B_2, B_6, vitamin A, vitamin E and vitamins of group F.

Foxtail millet can be grown in semi-arid areas as it tolerates drought and high temperatures, but it is very sensitive to frosts. It is highly tolerant to the poor, acidic and saline soils, and due to a short growing period (90-110 days), it can be grown at higher altitudes.

The production technology of the alternative cereals

Alternative cereals should be grown in crop rotation. The best preceedings are unrelated species after which the soil is not weedy and with good physical properties. The system of cultivation in crop rotation is of particular importance in organic farming since using a proper choice of preceeding we exclude the use of chemical plant protection products. The best preceedings are the legumes, perennial grass-legume mixtures, and all wide-rows crops of intensive cropping for which, besides the primary tillage, the organic nutrients were incorporated and performed intensive weed control.

To achieve high and stable yields, the soil tillage plays an important role, and the applied tillage system depends on the species. These cereals are crops of the spring planting and basic tillage can be done in the fall or early winter. Depth of plowing depends on the physical characteristics of the soil and the depth of the tillage for preceding crop, and usually it is performed at 20-25 cm.

Plant nutrition, as agrotechnical measure, is of great importance for the production of crops, because with optimal nutrition it will be the best use of natural fertility of the soil, growing conditions and genetic potential of the plant fertility. With supplementary feeding it is necessary to properly balance the amount and ratio of the major elements in nutrition. These values are determined based on knowledge of the natural fertility of soil, the plant need for nitrogen, phosphorus and potassium,

as well as of the coefficient of utilization of these elements. Table 1 shows the average amount of NPK nutrients for the proposed crops.

Table 1. The required amounts of NPK (kg ha^{-1})

Species	NPK amount
Buckwheat	60:110:70
Foxtail millet	80:60:40
Amaranth	20:30:20

Source: *Glamočlija et al.*, 2012

In plant nutrition it is used NPK mineral nutrients that can be incorporated into the soil during the basic tillage (half of them) an also before presowing preparation or with seeding. Along with them it may be used organic feed, manure, compost, etc. The crops feeding can be carried out using the mineral nutrient in the form of granules, or using the foliar application, together with the protection of crops. The effect of feeding is higher when the plants are grown in the irrigation system. In dry farming crop nitrogen fertilization is rarely applied.

Varieties: The program of breeding and the creation of new genotypes of the alternative cereals in the past is very important, because the needs of the food industry for these raw materials increasing. Varieties of the latest generation are characterized by productive organs, which, in addition to high nutritional value, also have significant amounts of vitamins, mineral salts and other useful nutritional substances. In Table 2 a suggestion of available and proven varieties of the mentioned plant species is given.

Table 2. -An overview of the major genotypes of millet-like cereals

Species	Varieties
Buckwheat	*Sivka, Golubica, Bogatyr Šatilovskaja 4,*
Foxtail millet	*Novosadski bar, Bučanskaja, Istrinskaja, Moskovskaja*
Amaranth	*A 10, Amont, Olpir, Plainsman,1008* (grain), *OMM, PI 482049* (biomass), *Green thumb, Pygmy* (decorative)

Source: *Glamočlija et al.*, 2012

Sowing: Seeding should be done with a good quality seeding material, which means that the seed should has a high germination (above 90%), purity above 95%, that it is uniform in size, varietal, with no admixture of weeds and healthy. These cereals are grown, depending on the species, at different spacing between the rows and spacing in the row, meaning, as wide-row and narrow-row crops (Table 3).

Table 3. -The ways of sowing of millet-like cereals

S p e c i e s	Plant spacing (cm)	Optimal plants number (per m^2)	Seeds amount (kg ha^{-1})
Foxtail millet*	24-50 densely	150-200	5-8
Buckwheat	12-50 densely	150-350	40-80
Amaranth*	50-70 h 20	10-150.000	2-4

*for production of grain
Source: *Glamočlija et al.*, 2012

Regarding the time of sowing, these cereals are spring crops. The minimal temperatures for germination of amaranth is 8-10^{0}C, so that the sowing of this species can be done at the beginning of April. Foxtail millet and buckwheat germinate at a temperature of 10-12^{0}C, so the planting should be done in the second half of April. These species have a short growing period (80-120 days) and can be successfully sown and later, for example after mowing of the winter forage mixtures.

The dense planting is best if it is done with vegetable seed drills for proper distribution of seeds in rows. If it is performed with wheat seed drills, small seeds (eg. amaranth) can be mixed with some inert material, such as ground corn, in the ratio of 3:1, in order to be better distributed on the surface. Soil before planting should be well prepared to be minced, moderately wet and heated in seeding layer. The depth of planting depends on seed size and it is 1-2 cm for small grain species, and up to 7 cm for gross grain species. If the surface layer is drained after the sowing, the field should be rolled with smooth rollers.

On smaller areas, such as the gardens, amaranth can be grown through seedlings. For this kind of method of growing, amaranth is sown in cold garden bed 30 days before the seedlings are brought out in the open field. The planting is done by hand on the surface on which the places had been marked previously with the pen. The production with seedlings has its advantages because the amaranth can be grown as subsequent or stubble crop.

Conclusion

Climatic and soil conditions in the agricultural areas of the Republic of Serbia provides the possibility of growing the alternative cereals such as buckwheat, foxtail millet and amaranth. These crops can be used as cattle feed because they do not require the special growing conditions and provide the yield that can quickly return the funds invested in their production.

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Mogućnost gajenja pojedinih alternativnih žita za ishranu stoke u klimatskim i zemljišnim uslovima Republike Srbije

R. Pivić, Đ. Glamočlija, D. Jošić, A. Stanojković Sebić ,Ž.Dželetović

Rezime

U ishrani domaćih životinja, uloga žita je vrlo značajna. Plodovi, zrna koriste se kao važne komponente za pripremu koncentrovane stočne hrane. Značaj u ishrani domaćih životinja pored ječma, ovsa, kukuruza, sirka, prosa i tritikalea, mogu imati i pojedina alternativna žita kao što su heljda, štir i čumiza čiji klimatski i zemljišni uslovi gajenja na području Republike Srbije odgovaraju uslovima rasta i razvića ovih biljnih kultura. Ostvareni prinosi mogu opravdati ulaganja u proizvodnju i za potrebe ishrane gajenih životinja.

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ANALYSIS OF SOME OF THE TRAITS THAT DETERMINE THE PRODUCTIVITY OF OATS IN ORGANIC AND CONVENTIONAL PRODUCTION

D. Spasova, D. Spasov, B. Atanasova, M. Ilievski

University „Goce Delcev"-Stip, Faculty of Agriculture, st. „Goce Delcev'', b.b. 2400 Strumica, Macedonia
Corresponding author: dragica.spasova@ugd.edu.mk
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Abstract: In the period 2005-2007 trials with five oats populations (*krivogastani,trebenista,radolista,bulgaria, kuceviste*) and three oats varieties (*rajac,slavuj, lovcen*), were carried out. The experiments were set in conditions of organic and conventional production. The main goal was to determine the differences in some of the traits that determine the productivity of oats, as well as differences that arise as a result of growing systems. Number of oat stalks in 1 m^2 in organic production (449) is greater absolutely for 20, or relatively for 4.5% of the number of stalks in 1 m^2 in conventional production (428). The number of panicles in 1 m^2 in organic production (445) is greater absolutely for 21or relatively for 4.8% from the number of panicles (424) in conventional production. Statistically reliable difference in number of stalks and panicles in 1 m^2 in organic and conventional production was not obtained in the tested varieties and populations. The production system of oats did not show a particular increase in the number of stalks in a panicle, although there is some increase in organic production, which was not going after a certain legitimacy to draw the right conclusions.

Key words: oat, stalk, panicle, node, organic, conventional

Introduction

Oats (*Avena sativa* L.) is a crop grown primarily for grain and straw. Oats as culture was introduced in Europe more than 3500 years ago and is considered as "European grain." It is thought that originats mainly from two types: wild oats (*Avena fatua* L.) and red wild oats (*Avena sterilis* L.), *Suttie JM (2000).* Area under oats over the world have been decreasing steadily, mainly due to lower and unstable yields in comparison with wheat and barley. As reasons for the low

productivity of oats are growing poorly productive varieties and improper agrotechnics. Poor scientific research in oats contributes to a series of unsolved agro-technical issues that greatly affect low production potential and reduction of area. On the other hand, today oats is among the many important crops in the human diet, with increased demand in modern cooking and food technology. It contains, soluble dietary fiber, mainly ß – glucans whose content varies from 2,5 – 6,5 %, Przulj N. et al. (1998). According fat content in grain (4-7%), oats deviates only place of corn. Among cereals, oats is the richest source of minerals: Ca (57 mg/100 g fruit), P (520 mg/100g/zrno), K (384 mg/100g fruit) and Fe. It contains vitamins B1, B2, B6, K1, E, *Savova T. et al. (2005)*. Today, in the developed world, great attention is paid on the healthy food. World tends to produce healthy food imposes the need and the Republic of Macedonia to conduct investigations in this direction and gain additional knowledge about the reaction of oat genotypes to applied agrotechnics.

The main objective was to determine differences in some of the traits that determine the productivity of oats, differences that occur as a result of farming systems, and in particular to distinguish the best varieties or populations of both types of production.

Material and Methods

The tests were performed in field conditions on the experimental field of Faculty of agriculture - Strumica, at the University “Goce Delchev", Stip, during the period 2005 to 2007. The laboratory tests were performed in the laboratories of the Faculty of Agriculture.

Five oats populations, of which four were domestic and one was introduced from Bulgaria (*krivogastani, trebenista, radolista, bugaria, kuceviste*) and three oats varieties from Serbia (*rajac, slavuj, lovcen*) were analyzed. Two experiments were set up. In both all the above-mentioned genotypes of oats were present, except that in one experiment all variants were set in terms of conventional production, and the other in terms of organic production. The experiments consisted of 8 variants in four repetitions, divided by the method of random block system, with the dimension of basic parcel of 5 m^2. The distance between the variants was 0,50 m, and between repetitions 1,0 m. The distance between rows was 20 cm. The seeding rate was 550 grains per 1 m^2. In the three years of testing the soil was prepared in an identical manner. The main treatment was plowing at a depth of 35 cm, followed by fertilization in methodological principle. In all the years of experiments, sowing is performed during March: 17.3.2005; 28.03.2006 and 06.03.2007, ie. when there were optimal conditions. Sowing is performed manually, in rows, at a depth of 5-6 cm.

Before harvest, material of 1 m^2 from each parcel is taken for laboratory analysis. In the laboratory the number of stalks and number of panicles in 1 m^2 were analyzed. The number of stalks in panicle and the number of nodes in panicle were analyzed on 30 plants of each parcel, ie. 120 plants of each variety.

The results are processed with statistical method for analysis of variance, and differences were tested by LSD-test.

Results and Discussion

Number of stalks in 1 m^2. When processing the results, we speak for the number of stalks in 1 m^2 because all plants are counted together, and are not counted separately with their tillers. The results for the number of stalks in the 1m^2 in conventional oats production are shown in Table 1, a in organic production in Table 2. When compared to the general averages of the number of stalks in 1 m^2 of both farming systems, regardless of years, genotype and climatic conditions, and depending on the applied agro-technical measures, it can be said that the number of stalks in 1 m^2 in organic production (449) is greater absolutely for 21 or relative for 4.9% than the number of stalks in 1 m^2 in conventional production (428). Increasing the number of stalks and the number of spikes in wheat affected by the fertilization has concluded *Vasilevski G. (1980).* Our results are consistent with results from the author. According*, Jevtić S. (1992),* during the growth of the stalk and in the tasseling stage, oats have the greatest need for nutrients. Regardless of year, climatic conditions and farming systems ie. applied agrotechnics, the best genotype of examined varieties and populations for the high number of stalks in 1 m^2 of oats is *radolista* population, which in the organic production system formed approximately 514 stalks in 1 m^2, and the system of conventional production 478.

Table 1. Number of stalks per 1 m^2 in conventional oat production

Variety/Population	Year			Average by variety/population 2005/07
	2005	2006	2007	
Krivogastani	429	417	419	422
Trebenista	472	384	474	443
Radolista	490	439	506	478
Bugaria	477	376	434	429
Kuceviste	464	368	455	429
Rajac	430	355	469	418
Slavuj	391	365	391	382
Lovcen	461	387	426	425
M	452	386	447	428
LSD 0,05	ns	ns	ns	
0,01	ns	ns	ns	

Table 2. Number of stalks per 1 m^2 in organic oat production

Variety/Population	Year			Average by variety/population 2005/07
	2005	2006	2007	
Krivogastani	479*	459	490	476
Trebenista	450	380	428	419
Radolista	516*	505	522	514
Bugaria	478*	440	469	462
Kuceviste	420	381	430	410
Rajac	358	416	458	411
Slavuj	393	445	432	423
Lovcen	461	450	505	472
M	444	435	467	449
LSD 0,05	108	ns	ns	
0,01	ns	ns	ns	

Number of panicles per 1 m^2. The results for the number 1 m^2 panicle in the conventional production of oats are shown in Table 3, and in organic production in Table 4. When compared to the general averages of number of panicles in 1 m^2 of both farming systems, regardless of age, genotype and climatic conditions, and depending on the applied agro-technical measures, it can be said that the number of panicles in 1 m^2 in organic production (446) is greater absolutely for 22 or relative for 5,2 % from the number of panicles in conventional production (424).

Table 3. Number of panicles per 1 m^2 in conventional oat production

Variety/Population	Year			Average by variety/population 2005/07
	2005	2006	2007	
Krivogastani	420	414	412	415
Trebenista	462	380	463	435
Radolista	482	430	500	471
Bugaria	475	375	430	427
Kuceviste	460	365	450	425
Rajac	430	352	468	417
Slavuj	390	360	390	380
Lovcen	455	384	422	420
M	452	386	447	424
LSD 0,05	ns	ns	ns	
0,01	ns	ns	ns	

Table 4. Number of panicles per 1 m^2 in organic oat production

Variety/Population	Year			Average by variety/population 2005/07
	2005	2006	2007	
Krivogastani	468*	450	504	474
Trebenista	435	380	488	434
Radolista	506*	497	515	506
Bugaria	470*	435	450	452
Kuceviste	412	380	430	407
Rajac	348	414	448	403
Slavuj	390	440	430	420
Lovcen	456*	447	501	468
M	436	430	471	446
LSD 0,05	104	ns	ns	
0,01	ns	ns	ns	

According to *Georgieva T. (1995),* the maximum general tillering at oats increases with increasing nitrogen fertilization, from 3.37 (at N_0), to 4.11 (at N_{18}), and productive tillers increase from 1.63 (at N_0), to 1.93 (at N_{18}). The highest percentage of the maximum established tillers develop into productive in the variant without nitrogen fertilization. According to the results obtained for the number of stalks and panicles in both farming systems can be stated that the number of panicles in all tested species and populations is proportional to the number of stalks in 1 m^2.

Number of stalks per panicle. The panicle in oats is composed of a central shaft (spindle) with an average of six nodes, *Vasilevski G. (2004)*. Lateral stalks of the panicle are developed on the nodes and on these panicles the spikelets are found. The results for the number of stalks per panicles in conventional oats production are shown in Table 5, a for organic production in Table 6. When compared to the general averages of the number of stalks per panicle in 1 m^2 of both farming systems, regardless of the year of production, genotype and climatic conditions, and depending on the applied agro-technical measures, it can be concluded that the number of stalks per panicle in 1 m^2 in organic production (22, 0) is higher absolutely for 0.7 or relative for 3,3 % than the number of of stalks per panicle in 1 m^2 in conventional production (21.3). The production system of of oats has not shown some increase in the number of stalks per panicle in 1 m^2, although there is some increase in organic production, but it does not go in a certain legitimacy to draw correct conclusions.

Table 5. Number of stalks per panicle in conventional oat production

Variety/Population	Year			Average by variety/population 2005/07
	2005	2006	2007	
Krivogastani	23.2	19.9	20.2*	21.1
Trebenista	21.6	22.2*	17.9	20.6
Radolista	22.6	20.1	19.1	20.6
Bugaria	22.5	21.3	18.7	20.8
Kuceviste	21.4	21.0	20.0	20.8
Rajac	23.0	22.4**	20.9	22.1
Slavuj	22.0	22.1*	21.5	21.9
Lovcen	23.4	22.9**	21.5	22.6
M	22.5	21.5	20.0	21.3
LSD 0,05	N.S.	1.77	1.72	
0,01	N.S.	2.56	2.49	

Regardless of year, climatic conditions and farming systems i.e applied agro-technical measures, the best genotype of examined varieties and populations for a number of of stalks per panicle in 1 m^2 of oats is variety *lovcen* which in the system of organic production reached an average number of of stalks per panicle in 1 m^2 of 23.6 and 22.6 in conventional production.

Table 6. Number of stalks per panicle in organic oat production

Variety/Population	Year			Average by variety/population 2005/07
	2005	2006	2007	
Krivogastani	23.3	21.8	21.5	22.2
Trebenista	23.1	20.2	17.9	20.4
Radolista	22.2	20.5	23.0**	21.9
Bugaria	22.7	22.1	19.5	21.4
Kuceviste	21.7	23.1	20.2	21.7
Rajac	23.3	24.0**	22.5**	23.3
Slavuj	22.1	224	21.6	22.0
Lovcen	25.4*	23.4*	22.2*	23.7
M	23.0	22.2	21.1	22.1
LSD 0,05	2.28	1.27	0.54	
0,01	ns	1.85	0.78	

Number of nodes per panicle. The results for the number of nodes per panicle in conventional oats production are shown in table 7, and in organic production in table 8.

Table 7. Number of nodes per panicle in conventional oats production

Variety/Population	Year			Average by variety/population 2005/07
	2005	2006	2007	
Krivogastani	5.6**	5.5**	5.1	5.4
Trebenista	5.3	5.1	4.9	5.1
Radolista	5.1	5.4	5.0	5.2
Bugaria	5.2	5.4	4.9	5.2
Kuceviste	5.3	5.3	5.2*	5.3
Rajac	5.6**	5.5**	5.1	5.4
Slavuj	5.5*	5.3	5.1	5.3
Lovcen	5.5*	5.5**	5.0	5.3
M	5.4	5.4	5.0	5.3
LSD 0,05	0.29	0.17	0.25	
0,01	0.42	0.24	ns	

Table 8. Number of nodes per panicle in organic oats production

Variety/Population	Year			Average by variety/population 2005/07
	2005	2006	2007	
Krivogastani	5.2	4.9	5.1	5.1
Trebenista	5.3	5.2	5.1	5.2
Radolista	5.4	5.3	5.2	5.3
Bugaria	5.3	5.2	5.0	5.2
Kuceviste	5.3	5.4*	5.1	5.3
Rajac	5.6*	5.3	5.0	5.3
Slavuj	5.7*	5.4*	5,3	5.5
Lovcen	5.6*	5.4*	5.1	5.4
M	5.4	5.3	5.1	5.3
LSD 0,05	0.34	0.34	ns	
0,01	ns	ns	ns	

The production system of oats showed no increase in the number of nodes per panicle, meaning this property showed high stability. Regardless of year of production, climatic conditions and farming systems i.e. applied agrotechnic, the best genotype of examined varieties and populations for the number of nodes per panicle is the variety *rajac*, which in organic and conventional production reached an average of 5.4 nodes per panicle. According to Finker et al. (1973) quoted by Milnar R. et al (1996), the lower and early maturing oat varieties often have fewer nodes per panicle (fertile layers), which reduces the number of spikelets and yield of grain by panicle.

Conclusion

Based on three years of research, the following conclusions can be drawen: The number of stalks in 1 m^2 in organic production (449) is greater absolutely for 21or relative for 4.9% of the number of stalks in 1 m^2 in conventional production (428). The best genotype of examined varieties and populations for the high number of stalks in 1 m^2 of oats is *radolista* population, which in both farming systems reached the highest number of stalks per 1 m^2 (514 in organic production and 478 in conventional production). Population in organic production reached an average number of 506 panicles and in conventional production 471. The number of stalks per panicles does not depend on the length of the panicle, but the density of the panicle and the number of the nodes per panicle, on which the lateral stalks are developed. Varieties with condensed panicle have many stalks in the panicle. The most stalks per panicle in both farming systems made variety *lovcen*, which in organic production reached an average number of stalks per panicle of 23.6 i.e. 22.6 in conventional production. All varieties grown in the organic production system had higher average number of spikelets, absolutely for 1.5 or relatively for 2.1% than the number of spikelets in conventional production. The production system of of oats has not shown some increase in the number of stalks per panicle in 1 m^2, although there is some increase in organic production (22.0) absolutely for 0.7 or relatively for 3.3% than the number of stalks per panicle in conventional production (21.3). The number of nodes per panicle is a variety characteristic, but it depends on the climatic conditions of the year. Lowest number of nodes per panicle in both farming systems were obtained in the year of 2007, as the most arid in thein the initial stages of development of oats. Best genotype of the tested varieties and populations for a number of nodes per panicle is variety *rajac*, which in organic and conventional production reached an average number of nodes per panicle of 5.4

Analiza nekih osobina koje određuju produktivnost ovsa u organskoj i konvencionalnoj proizvodnji

D. Spasova, D. Spasov, B. Atanasova, M. Ilievski

Rezime

U periodu 2005-2007, postavljeni su ogledi sa pet populacija ovsa (krivogastani, trebenista, radolista, bugarska, kučevište) i tri sorte ovsa (Rajac,

Slavuj, Lovćen), su sprovedene. Eksperimenti su postavljeni u uslovima organske i konvencionalne proizvodnje.Osnovni cilj je bio da se utvrde razlike u nekim od osobina koje određuju produktivnost ovsa, kao i razlike koje se javljaju kao rezultat rastućih sistema. Broj ovsenih stabljika u 1 m^2 u organskoj proizvodnji (449) je apsolutno veći za 20, ili relativno za 4,5% od broja stabljika u 1 m^2 u konvencionalnoj proizvodnji (428). Broj metlica u 1 m^2 u organskoj proizvodnji (445) veći apsolutno za 21 ili relativno za 4,8% od broja metlica (424) u konvencionalnoj proizvodnji. Statistički pouzdane razlike u broju stabljika i metlica u 1 m^2 u organskoj i konvencionalnoj proizvodnji nije utvrđena u ispitivanim sortama i populacijama. Sistem proizvodnje ovsa nije pokazao određeni porast broja stabljika i metlica, iako postoji povećanje u organskoj proizvodnji.

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THE INFLUENCE OF SOWING DENSITY, COMPACTION DEGREE AND INOCULATION ON CHEMICAL COMPOSITION AND QUALITY OF MAIZE SILAGE

R. Dubljević[1], N. Đorđević[2], M. Damjanović[2], D. Mitrović[2]

[1]Biotechnical Faculty, University of Montenegro, Mihaila Lalića 1, 81000 Podgorica, Montenegro
[2]Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Zemun, Republic of Serbia
Corresponding author: ratod@ac.me dubljevici@t-com.me
Original scientific paper

Abstract: Experiment was carried out according to statistical model 2×2×2, where factor A is sowing density (A_1 = 60,000 plant/ha and A_2 = 75,000 plant/ha), factor B is the degree of compactness of silage (B_1 = 700 g/dm^3, B_2 = 600 g/dm^3) and C is inoculation of the silage material (C_1 = without inoculants; C_2 = with inoculants). All whole maize plant silages after the treatment were compressed in plastic experimental siloses with volume of 60 dm^3 . After 90 days the experimental siloses were opened and representative samples were taken for chemical analyses. Based on chemical analysis it has been determined that in silage that was more compacted (700 g/dm^3) amount of lactic acid has increased ($P<0.05$). The silage inoculation intensified the fermentation and lessened the pH value ($P<0.01$). Influence of sowing density was not significant for parameters of the chemical composition and silage quality. On the basis of this investigation it may be concluded that the degree of compression is the most important parameter in ensiling technology. With the adequate compression and reduction of air in the starting material, the aerobic phase is reduced and the dry matter losses are reduced.

Key words: maize, silage, sowing density, compactness, inoculation, chemical composition, quality.

Introduction

The importance of maize as a crop species for animal and human nutrition is significantly increased with the spreading of ensiling conserving technology of the whole plant. Whole plant maize silage is one of the most important energy sources in cattle feeding, considering the high green mass yields, relatively high energy content, high palatability and its importance in total mixed ration (TMR)

formulation *(Forouzmand et al., 2005)*. Lately maize silage is becoming an important winter feed for sheep as well as goats, however some authors have pointed out possible problems in its usage for small ruminants *(Memiši and Bauman, 2007)*. Today great attention is given not just to the silage quality, but to its nutritive value which depends on many factors.

Nutritive value of maize silage is defined by hybrid, degree of maturity and dry matter digestibility *(Kamalak et al., 2003)*. Modern maize hybrids have such habitus that allows them to be grown in more dense rows, which allows higher yield *(Subedi et al., 2006)*. Increase of crop density in process of growing the silage maize is justified only from aspect of yield increase per acre, only if nutritional value of produced silage is not changed *(Budakli et al., 2010)*. Besides nutritional value, silage quality is highly influenced by degree of compactness (the degree of anaerobiosis). In anaerobic conditions lactic bacteria are dominant because they are partly anaerobic and they produce mainly lactic acid. However for maize silage it is desirable that next to lactic acid (produced by bacteria), there are minor amounts of acetic and butyric (fungicidal) acid. These acids protect the maize silage from fungi, yeasts and molds after opening of the silo. Therefore nowadays inoculation of maize silage is done with inoculates that are based on combination of homo and hetero-fermentative bacteria of lactic acid *(Đorđević et al., 2012a)*.

In Montenegro, lately, silage is being made more frequently and is used in cattle diet. Maximal compactness of biomass and fermentation stimulation is very important for quality parameters and silage usability. With that in mind this experiment was planned, its aim was to examine the influence of sowing density, compaction degree and inoculation on chemical composition and quality of maize silage.

Materials and methods

Experiment was carried out according to statistical model 2×2×2, where factor A is sowing density (A_1 = 60,000 plant/ha and A_2 = 75,000 plant/ha), factor B is the degree of compactness of silage (B_1 = 700 g/dm^3, B_2 = 600 g/dm^3) and C is inoculation of the silage material (C_1 = without inoculants; C_2 = with inoculants). Silage was made in maize waxing phase. Prepared biomass was treated with inoculants based on lactic acid homo- and heterofermentative bacteria in order to intensify the fermentation. All whole maize plant silages after the treatment were compressed in plastic experimental silos with volume of 60 dm^3. After 90 days the experimental silos were opened and representative samples were taken for chemical analyses.

Chemical composition and silage quality were analyzed in the Laboratory for nutrition of domestic animals on the Faculty of Agriculture, Zemun *(AOAC,*

2002). Statistical analyze of obtained results was done with analysis of variance procedure with software package Statistica v.6. (Statsoft, 2006).

Results and discussion

The amount of dry matter in all silages was above 300 gkg^{-1} which is considered as the main condition to prevent separation of juices and maximal control of butyric fermentation (Table 1). Difference in chemical composition of the silage between A_1 and A_2 treatments were minimal and can be explained, mainly with influence of sowing density to certain parts of the plant (leaf, stem, cob). Highest part of nutritive value originates from the cob, whose percent is less if crop density is too high *(Đorđević and Dinić, 2007)*. Most significant differences are differences in total amount of dry matter and energy, between different sowingdensity *(Budakli et al., 2010)*.

Table 1. Chemical composition of silages, g/kg DM

Treatments			DM g/kg	Proteins	Fat	Cellulose	NFE	Ash
Sowing density	Degree of compactness	Inoculant						
(A_1) 60,000 plant/ha	(B_1) 700 g/dm^3	(C_1) Without	298.72	70.64	27.79	193.51	669.90	38.17
		(C_2) With	281.60	70.67	30.54	182.53	672.94	43.32
	(B_2) 600 g/dm^3	(C_1) Without	360.79	76.22	27.16	152.44	715.08	29.10
		(C_2) With	357.41	80.58	26.86	182.23	659.76	47.57
(A_2) 75,000 plant/ha	(B_1) 700 g/dm^3	(C_1) Without	319.10	72.70	26.64	214.35	652.46	33.85
		(C_2) With	311.18	70.37	25.39	206.30	656.81	41.13
	(B_2) 600 g/dm^3	(C_1) Without	338.83	74.68	22.43	174.44	689.79	38.67
		(C_2) With	334.16	73.93	31.13	152.35	706.67	35.92
Average for A_1			324.63	74.53	28.09	178.43	679.42	39.54
Average for A_2			325.82	72.92	26.40	186.86	676.43	37.39
Average for B_1			302.65	72.00	27.59	199.17	663.03	39.12
Average for B_2			347.80	76.35	26.70	166.12	692.82	37.82
Average for C_1			329.36	73.56	26.00	183.68	681.81	34.95
Average for C_2			321.09	73.89	28.48	181.60	674.04	41.98
Significance for A			ns	ns	ns	ns	ns	ns
Significance for B			**	ns	ns	*	*	ns
Significance for C			ns	ns	ns	ns	ns	*

ns – no significance; * ($p<0.05$); ** ($p<0.01$)

Inoculants were used in order to provide the optimal number of lactic acid bacteria, which are usually in very small numbers in nature. *Jambor and Šiške (1997)* concluded that number of epiphyte lactic acid bacteria on plants is only 10-10^2 per gram of green mass, while number of enterobacteriace is much higher 10^2-10^7 per gram. Commercial products used as inoculants are used with the doses that

provide 10^5-10^6 lactic acid bacteria per gram of ensiled mass, which is enabling them to become dominant over the enterobacteria *(Đorđević et al., 2012b)*. Maize is one of the plant species that are easily ensiled, due to its abundance of fermentable carbohydrates. This is why in its ensiling it is unnecessary (and even harmful) to use homofermentative bacterial inoculants to enhance lactic acid fermentation. *(Đorđević et al., 2007)*. Because of the shorter duration of fermentation and high amount of sugars that remain unfermented, such silage is perfect substrate for the activity of aerobic microorganisms when the silo is opened *(Arriola et al., 2011)*. Low aerobic stability of maize silage is one of the largest problems with that feedstuff. It is occurring when silage is not used regularly and remains on the open air for longer periods of time. But, in experiment biomass was treated with inoculants based on lactic acid homo- and heterofermentative bacteria. Lactic acid, as the main product of homofermentative processes of sugar fermentation in the ensiled mass, is a strong anti bacterial but weak fungicide agents. Contrary to that, acetic, propionic and butyric acid in maize silages are desirable to some degree *(Hu et al., 2009)*. Inoculants in this experiment have intensified the fermentation and increased the production of lactic and acetic acid (Table 2). Result that came out of that was decreasing of the pH value in the inoculated silage.

Table 2. Parameters of biochemical changes in silages, g/kg DM

Hybrid	Degree of compactness	Inoculant	pH	Lactic acid	Acetic acid	Butyric acid
(A_1) 60,000 plant/ha	(B_1) 700 g/dm^3	(C_1) Without	3.80	33.49	12.39	0,00
		(C_2) With	3.69	49.72	34.09	0,00
	(B_2) 600 g/dm^3	(C_1) Without	3.65	34.09	14.14	0,00
		(C_2) With	3.86	48.41	24.06	0,00
(A_2) 75,000 plant/ha	(B_1) 700 g/dm^3	(C_1) Without	4.09	30.08	5.64	0,00
		(C_2) With	3.54	49.16	20.24	0,00
	(B_2) 600 g/dm^3	(C_1) Without	3.78	37.78	14.76	0,00
		(C_2) With	3.64	48.38	21.85	0,00
Average for A_1			3.75	41.42	21.85	0,00
Average for A_2			3.76	41.35	21.17	0,00
Average for B_1			3.78	33.11	15.62	0,00
Average for B_2			3.73	41.35	18.09	0,00
Average for C_1			3.83	33.86	11.73	0,00
Average for C_2			3.68	48.92	25.06	0,00
Significance for A			ns	ns	ns	-
Significance for B			ns	*	ns	-
Significance for C			**	**	**	-

ns - no significance; * ($p<0.05$); ** ($p<0.01$)

In silage that had higher level of compactness the lactic acid content was significantly higher also there was significantly lower amount of acetic acid.

Explanation for that is that oxidative phase is shorter when silage material is compacted better; therefore the production of acetic acid is less.

It was discovered that butyric acid was not present in silages. That can be explained with the adequate conditions during the ensiling, and the fact that raw material was not contaminated with soil, which is the main source of butyric *Clostridia (Đorđević et al., 2004)*. The explanation is also in the higher dry matter content in all starting materials, which is limiting the activity of butyric bacteria.

Silage quality was evaluated according to Flieg method. Flieg method gives points for relative ratio of lactic, acetic and butyric acid. All maize plant silages were ranked as first class (except treatment $A_1B_1C_2$). Based on the quality evaluation, all silages were useful for animal consumption.

Table 3. Relative ratio of acids (%) and silage quality

Hybrid	Degree of compactness	Inoculant	Relative ratio of acids, %			Points	Class by Flieg
			Lactic	Acetic	Butyric		
(A_1) 60,000 plant/ha	(B_1) 700 g/dm^3	(C_1) Without	72.99	27.01	0.00	95	I
		(C_2) With	59.32	40.68	0.00	80	II
	(B_2) 600 g/dm^3	(C_1) Without	70.68	29.32	0.00	95	I
		(C_2) With	66.80	33.20	0.00	88	I
(A_2) 75,000 plant/ha	(B_1) 700 g/dm^3	(C_1) Without	84.21	15.79	0.00	100	I
		(C_2) With	70.84	29.16	0.00	95	I
	(B_2) 600 g/dm^3	(C_1) Without	71.91	28.09	0.00	95	I
		(C_2) With	68.89	21.11	0.00	93	I

Conclusion

In this experiment we have determined significant influence of compaction level and inoculation to main biochemical parameters (pH value, amount of lactic and acetic acid). Chosen sowing density as one of the researched factors haven't had significant influence on chemical composition and quality of the silage.

On the basis of this investigation it may be concluded that the degree of compression is the most important parameter in ensiling technology. In practice the special attention should be given to factors on which directly or indirectly depends the level of compression on ensiled material: wilting, cutting, object selection and/or selection of machines used for compression.

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Uticaj gustine setve, stepena sabijanja i inokulacije na hemijski sastav i kvalitet silaža kukuruza

R. Dubljević, N, Đorđević, M. Damjanović, D. Mitrović

Rezime

Eksperiment je izveden po modelu 2×2×2, gde je A gustina setve (A_1 = 60.000 biljaka/ha i A_2 = 75.000 biljaka/ha), faktor B je stepen sabijenosti silaže (B_1 = 700 g/dm^3, B_2 = 600 g/dm^3) i C je inokulacija siliranog materijala (C_1 = bez inokulanta; C_2 = sa inokulantom). Sve silaže cele biljke kukuruza su nakon tretiranja sabijene u plastične eksperimentalne sudove zapremine 60 dm^3. Posle 90 dana po siliranju eksperimentalni sudovi su otvoreni i uzeti reprezentativni uzorci za hemijsku analizu.

Na osnovu hemijske analize, u silažama sa većim stepenom sabijenosti (700 g/dm^3) utvrđena je veća količina mlečne kiseline ($p<0,05$). Inokulacija silaža je intenzivirala fermentaciju i smanjila pH vrednost ($p<0,01$). Gustina setve nije bila značajna za parametre hemijskog sastava i kvaliteta silaža.

Na osnovu istraživanja može se zaključiti da je stepen sabijenosti najvažniji faktor za tehnologiju siliranja. Uz adekvatno sabijanje i redukciju vazduha u početnom materijalu, skraćuje se trajanje aerobne faze i time se smanjuju gubici suve materije.

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INFLUENCE OF INOCULATION ON GRAPE POMACE SILAGE QUALITY SUPPLEMENTED WITH NON-PROTEIN NITROGEN

V. Maraš[1], B. Gašović[1], D. Drakić[1], J. Raičević[1], J. Djaković[1], V. Kodzulović[1], S. Šucur[1], N. Djordjević[2]

[1]13. Jul Plantaže Podgorica, Put Radomira Ivanovića 2, 81000 Podgorica, Montenegro
[2]Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Zemun, Serbia
Corresponding author: vesnam@t-com.me
Original scientific paper

Abstract: In this work we investigated the influence of inoculation on quality of grape pomace silage with non-protein nitrogen addition (commercial source of non-protein nitrogen with 70% urea - NPN). The inoculation was done with inoculant based on a combination of homo and heterofermentative lactic acid bacteria. The experiment was done as two-factorial (4×2; n=3), where factor A was commercial source of non-protein nitrogen (A_1=control; A_2=with 10 gkg^{-1}; A_3=with 20 gkg^{-1}; A_3=with 30 gkg^{-1}), while factor B was inoculation (B_1=without inoculant; B_2=with inoculant). All silages after the treatment were compressed in plastic experimental silos with volume of 100 dm^3. After 150 days the experimental silose were opened and representative samples were taken for chemical analyses.

The differences in chemical composition between starting material and silages occurred because of the drying process of the samples during which some ammonia was lost. The use of NPN had significant influence on some parameters of chemical composition and biochemical changes in grape pomace silages. On treatments with NPN there was significant increase in crude protein and ammonia nitrogen content and pH value ($p<0.05$). With the inoculation of the ensiling material the production of lactic acid was reduced but content of butyric acid and ammonia nitrogen was increased. The changes were significant according to DLG silage scoring systems, so that silages without NPN were ranked as first class and silages with 30 gkg^{-1} NPN were ranked as fifth class.

Key words: grape pomace, silage, non-protein nitrogen addition, inoculation.

Introduction

Due to terrain and climate specificity, in Montenegro are for decades evident deficits in forage balance, which has negative impacts on the overall livestock production. For this reason, there is a great interest of farmers for the use of the following products in alcohol industry and fermentation in domestic animals feed, considering the existence of significant domestic capacities of this species. The practice of using following products from different types of food industry exists for the centuries (*Đorđević and Dinić, 2007; Mirzaei-Aghsaghali and Maheri-Sis, 2008*). The greatest tradition have the accompanying products of the milling industry and with its high protein content today the most important are the accompanying products of oil industry (*Đorđević et al., 1998*). The company ”13. jul Plantaže” (Podgorica, Montenegro) after the harvest and grape processing produces huge pomace quantities.

Based on quantitative and qualitative features, this kind of product can be interesting and important energetic food, especially in areas with less choice of food for ruminants. However, mentioned product contains high percentage of moisture, and because of that it can be used in short period of time, after which come the aerobic microbiological process, spoilage and inapplicability (*Zalikaranab et al., 2007; Bahrami et al., 2010*). In previous decades there was the practice of drying this feed (*Pirija et al., 1976*), what is today not feasible procedure, due to high energy prices. Because of that, surely the most current conservation is by ensiling of this and other feeds with high percentage of moisture.

This experiment was planned with aim to investigate the possibility of ensiling grape pomace with added bacterial inoculant (in order to intensify and direct fermentation of lactic acid type) and with commercial supplement based on urea (in order to increase crude protein content).

Material and Methods

In the experiment the fresh grape pomace of variety Rkaciteli is ensiled with the addition of commercial sources of non-protein nitrogen (NPN) with 70% urea and inoculants based on a combination of homo (*Lactobacillus plantarum* and *Enterococcus faecium*) and heterofermentative (*L. brevis* and/or *L. Kefiri*) lactic acid bacteria. The experiment was conducted by two factorial model, where the first factor (A) was the amount of commercial sources of non-protein nitrogen (A_1 = control, A_2=10 gkg^{-1}; A_3=20 gkg^{-1}; A_4=30 gkg^{-1}), and factor B inoculation (B_1=without inoculant; B_2=with inoculants, $4gt^{-1}$). Each treatment was performed

in three replicates (three separate experimental court) in order to process statistical analysis of results. Thus the obtained experiment type was 4×2, n=3. Plastic barrel with volume of 100 dm^3 were used for silage, which were closed with a screw caps. Grape pomace was ensiled immediately after receiving from the presses in the microvinification winecellar of ”13. Jul Plantaže” Podgorica-Montenegro. The compression degree was the same for each barrel, of about 100 gdm^{-3}, which is considered optimal for the creation of anaerobic conditions from the beginning.

Samples for laboratory analysis in order to determine the nutritional value and quality of silage were taken 150 days after ensiling. Chemical analysis of silage samples of grape pomace was performed in the laboratory of animal nutrition at the Agricultural faculty of the University of Belgrade. The parameters of the chemical composition were determined according to *AOAC (2002)* methods, the amount of lactic, acetic and butyric acids were determined by distillation method according to *Wiegner (1926)*, the amount of ammonia nitrogen by a modified Kjeldahl's method *(Dulphy and Demarquilly, 1981)*. The quality and usage of silage was evaluated using the *DLG (1987)* methods. Statistical analysis was performed by software *Statsoft (2006),* where the analysis of variance examined the significance of the factors, and for the significance of the factor interaction between treatments Tukey test was used.

Results and Discussion

Starting material of grape pomace from variety Rkatsiteli contained less protein level comparing to *Pirmohamaddi et al. (2007)*, i.e. less protein and cellulose content and more nitrogen-free extract (NFE) comparing to *Mirzaei-Agsaghali et al. (2011)*, what can be interpreted by influence of the year (low precipitation, high concentration of sugar in grape and therefore higher remains of sugar in pomace and relative change of other substances in feed). Compared to the starting material, all silages had a few percentages higher moisture level, what is explained by the influence of added water as solvent for used inoculant, but also because of partially loss of volatile substances during the sample drying at 105ºC, in order to determine the dry matter content. In all silages the determined dry matter content was higher than 300 gkg, by which was initially prevented juices extraction and reached maximum control of butyric type fermentation (Table 1).

Increase of all nutrients of organic and inorganic nature (with exception of crude proteins in the treatment A_1B_2 and nitrogen-free extracts) is determined in all investigated silages comparing to the content in starting material, it can be explained as consequence of volatiles loss during drying, and thus change of relative ratio of certain components.

Table 1. Chemical composition of starting material and silages, gkg^{-1} DM

Treatment		Dry matter, gkg	Crude proteins	Crude lipids	Crude fiber	NFE	Ash
Starting material							
		400.60	103.84	93.11	216.92	541.69	44.43
Silages							
A_1	B_1	336.17	104.23	101.00	274.61	461.44	58.72
	B_2	346.50	102.15	105.54	302.14	431.68	58.49
A_2	B_1	338.13	130.72	104.39	257.06	431.32	76.51
	B_2	320.53	145.98	103.59	240.84	427.83	81.76
A_3	B_1	335.00	161.28	105.49	251.84	393.51	87.88
	B_2	338.97	155.68	102.95	243.09	404.17	94.11
A_4	B_1	337.10	193.60	92.16	236.32	370.18	107.73
	B_2	330.53	212.99	92.68	273.02	321.05	100.27
Average for A_1		341.33	103.19a	103.26b	288.37c	446.56c	58.60a
Average for A_2		329.33	138.35b	103.99b	248.95a	429.58c	79.14b
Average for A_3		336.98	158.47c	104.22b	247.47a	398.84b	91.00c
Average for A_4		333.82	203.30d	92.42a	254.67a	345.61a	104.00d
Average for B_1		336.60	147.45A	100.76	254.96	414.11 B	82.71
Average for B_2		334.13	154.20B	101.18	264.77	396.18 A	83.66

A,B,a,b,c,d = Values in the same colon, for different factors and with different letters are significantly different ($p<0.05$)

The crude proteins level was steadily increasing in all silages, in accordance with the increase of commercial addition dose of non-protein nitrogen based on urea ($p<0.05$). Comparing to starting material, the quantity of crude proteins was increased for 100% in A_4B_1 and A_4B_2 treatments, as compared to silages A_1B_1 and A_1B_2, which were without addition. It si explained by the fact that urea contains 42% of nitrogen, which is equivalently for 263% of proteins.

The amount of crude fat in A_1B_1, A_1B_2, A_2B_1, A_2B_2, A_3B_1 and A_3B_2 was higher comparing to starting sample, which can be explained as consequence of relative increase of fat due to the loss of volatiles, but also as a result of lactic acid extraction (as non-volatile) by diethyl-ether, that is used for determination of crude fat amount according to Soxlet (*Đorđević et al., 2003*). The amount of crude cellulose (structural carbohydrates) was few percentages higher in all treatments than in starting material, what is also consequence of relative change of certain components. Crude cellulose amount in this experiment was considerably lower compared to quotes of *Nikolić et al. (1980).*

The amount of nitrogen-free extracts (non-structural carbohydrates, in this case sugars), was reduced in all silages, in comparison with the starting material, what is a result of their consumption in the process of microbiological fermentation, and also because of increase of commercial addition of non-protein nitrogen based on urea.

Evident increase in the amount of mineral matters in silages, compared to the starting material is consequence of increase of commercial addition percentage share of non-protein nitrogen, which basicaly contains aluminosilicates.

Starting material of grape pomace had low pH value (pH=4.02) which initially secured excellent conditions for lactic acid fermentation (Table 2). However, in treatments with NPN compound addition, it is noticable significant increase of pH value, what is consequence of basic character of the additive based on urea ($p<0.05$).

Table 2. Parameters of biochemical changes in silages (gkg^{-1} DM)

Treatment		pH	NH_3-N, gkg^{-1} N	Lactic acid	Acetic acid			Butyric acid		
					Free	Bonded	Total	Free	Bonded	Total
Starting material										
		4,02	-	-	-	-	-	-	-	-
Silages										
A_1	B_1	3.77	55.90	34.44	6.42	0.57	6.99	0.00	0.00	0.00
	B_2	3.81	71.34	34.87	3.50	0.64	4.14	0.00	0.00	0.00
A_2	B_1	5.06	422.89	27.55	3.91	10.93	14.84	0.00	0.00	0.00
	B_2	5.30	420.89	18.69	2.73	5.56	8.29	0.52	6.10	6.62
A_3	B_1	5.35	425.05	9.87	0.36	7.95	8.31	0.00	0.00	0.00
	B_2	5.68	404.25	5.84	0.00	9.59	9.59	0.00	0.39	0.39
A_4	B_1	6.83	398.26	5.34	0.00	7.01	7.01	0.00	0.00	0.00
	B_2	6.69	357.17	5.45	0.00	10.10	10.10	0.00	0.41	0.41
Average for A_1		3.79a	63.62a	34.67c	4.96c	0.60a	5.56a	0.00a	0.00a	0.00a
Average for A_2		5.18b	421.89b	23.12b	3.32b	8.24b	11.56b	0.26b	3.05b	3.31b
Average for A_3		5.51c	414.65b	7.86a	0.18a	8.77b	8.95ab	0.00a	0.19a	0.19a
Average for A_4		6.76d	377.70c	5.40a	0.00a	8.56b	8.56ab	0.00a	0.20a	0.20a
Average for B_1		5.25A	325.52B	19.31B	2.68B	6.61	9.23	0.00A	0.00A	0.00A
Average for B_2		5.37B	313.42A	16.22A	1.56A	6.47	8.03	0.13B	1.72B	1.85B

A,B,a,b,c,d = Values in the same colon, for different factors and with different letters are significantly different ($p<0.05$)

The amount of ammonia nitrogen increased in line with increasing of commercial supplement dose of non-protein nitrogen, from 55.90 gkg^{-1} N (A_1B_1) to 425.05 gkg^{-1} N (A_3B_1), what is about 750% ($p<0.05$). Ammonia nitrogen in the silage is the main indicator of protein degradation and results of proteolytic enzymes activity from plant cells and microorganisms, in the first place from butyric acid clostridia. The presence of ammonia in silages containing no butyric acid is result of the action of enzymes in plant (*McDonald et al., 1991*). In silages

of grape pomace with commercial additive of non-protein nitrogen, ammonia nitrogen is result of urea hydrolysis ($CO(NH_2)_2$) on CO_2 and $2NH_3$.

The largest amounts of lactic acid (absolute and relative) were found in treatments A_1B_1 and A_1B_2 (table 3). However, in subsequent treatments it comes to decreasing of lactic acid production, which can be explained by inadequate (high) pH values (reduction of absolute amounts) as well as specificities of heterotrophic bacteria in inoculants (decrease in relative amounts). In treatments A_4B_1 i A_4B_2, minimal amounts of lactic acid were determined due to reduced activity of LAB bacteria in unfavorable conditions and due to consumption of already formed lactic acid by butyric acid clostridia. When increasing doses of NPN supplement, it led to decreasing of amounts of free acids and increasing of linked acids which contribute to increasing of pH values. In treatments A_3B_1, A_3B_2, A_4B_1 and A_4B_2 practically were not free acetic and butyric acid because they were linked by aluminosilicates from NPN supplement.

In all treatments, with the simultaneous addition of comercial products of non-protein nitrogen butyric acid was detected, whose participation in treatments A_2B_2 was 19,70% in total acidity (Table 3). Such large amounts can cause ketosis in ruminants and they are absolutely unacceptable (*Đorđević et al., 2012*). In the other treatments (A_3B_2 and A_4B_2) share of butyric acid was less than 2,6% of the total share acids and it can be considered „harmless" to the health of ruminants. The emergence of butyric acid in this case is explained by a high pH value. For example, the main condition for butyric clostridia activity mentioned in the literature is moisture more than 700 g/kg as well as pH values higher than 4,5 (*Đorđević and Dinić, 2003*).

For silage quality assessment it was used DLG method which considers pH value and percentage of total lactic, acetic and butyric acid. According to the used method, silages without addition of commercial source of nitrogen based on urea were evaluated as I class, regardless of inoculants. When using the commercial source of non-protein nitrogen based on urea (1%) it led to decreasing of silage quality on II class (without inoculants) or III class (with inoculants), when using 2% of addition, silage quality was decreased on III class (without inoculants) or IV class (with inoculants) and using 3 % of supplement, silage quality is evaluated as IV class (without inoculants) or V class (with inoculants).

Table 3. Relative ratio of acids and quality class by DLG method

Treatment		Relative ratio of acids, %			Points	Class by DLG
		Lactic	Acetic	Butyric		
A_1	B_1	83.13	16.87	0.00	49	I
	B_2	89.39	10.61	0.00	50	I
A_2	B_1	64.99	35.01	0.00	43	II
	B_2	55.63	24.67	19.70	36	III
A_3	B_1	54.29	45.71	0.00	36	III
	B_2	36.92	60.62	2.46	23	IV
A_4	B_1	43.24	56.76	0.00	25	IV
	B_2	34.15	63.28	2.57	17	V

Conclusion

Grape pomace represents suitable material for ensiling considering the fact that it contains a lot of fermentable carbohydrates which ferment and give lactic acid with more than 80% in total content of acids. The use of selected inoculants of this material proved to be unnecessary since it results in a further reduction of the percentage fraction of acetic acid, which can worsen the aerobic stability of silage. The use of a commercial source of non-protein nitrogen based on urea, in aim of „breeding" grape pomace silage with crude proteins, at concentration of 1, 2 and 3% in this experiment proved even harmful. Beside the increase in the proportion of ammonia nitrogen, use of this type of supplement has led to a significant increase in pH, as well as the modification of fermentation in terms of building large quantities of acetic and butyric acids. Based on this experiment, it is recommended for future research the use of small amounts of add-commercial sources of non-protein nitrogen based on urea, about 0.5% or less. When using pomace silage in feeding ruminants in order to correct nutritional value (a small amount of crude protein and large amount of energy matter) it is recommended to supplement voluminous part of the meal with proteins of plant origin (alfalfa hay), than to supplement a concentrated part with meal with proteins of plant origin (soybean or sunflower meal) or use of some commercial source of non-protein nitrogen based on urea also in concentrated part of meal.

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Uticaj inokulacije na kvalitet silaže komine grožđa sa dodatkom neproteinskog azota

V. Maraš, B. Gašović, D. Drakić, J. Raičević, J. Djaković, V. Kodzulović, S. Šucur, N. Djordjević

Rezime

U radu je ispitivan uticaj inokulacije na kvalitet silaže komine grožđa sa dodatkom neproteinskog azota (komercijalni izvor neproteinskog izvora azota sa 70% uree - NPN). Za inokulaciju je korišćen inokulant na bazi kombinacije homo i heterofermentativnih bakterija mlečne kiseline. Eksperiment je urađen kao dvofaktorijalni (4×2, n=3), gde je A bio komercijalni izvor neproteinskog azota (A_1=kontrola; A_2=10 gkg^{-1}; A_3=20 gkg^{-1}; A_3=30 gkg^{-1}), a faktor B je inokulacija (B_1=bez inokulanta; B_2=sa inokulantom). Sve silaže su nakon tretiranja dodacima sabijene u plastične eksperimentalne sudove zapremine 100 dm^3. Posle 150 dana otvoreni su eksperimentalni silosi i uzeti su reprezentativni uzorci za hemijske analize.

Razlike u hemijskom sastavu između početnog materijala i silaža su posledica sušenja uzoraka, pri čemu je došlo do gubitka dela amonijaka. Upotreba NPN značajno je uticala na neke parametre hemijskog sastava i biohemijskih promena u silažama komine grožđa. U tretmanima sa NPN dodatkom došlo je do značajnog povećanja količine sirovog proteina, amonijanog azota i pH vrednosti ($p<0,05$). Pri inokulaciji siliranog materijala došlo je do redukcije proizvodnje mlečne kiseline, dok je sadržaj buterne kiseline i amonijačnog azota povećan. Promene su bile značajne za DLG metodu ocene kvaliteta, pa su silaže bez NPN ocenjene I klasom kvaliteta a silaže sa 30 gkg^{-1} NPN rangirane su u V klasu kvaliteta.

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IN VITRO MYCOTOXINS ADSORPTION BY SOUR CHERRY STONES

Z. Lopičić[1], A. Bočarov–Stančić[2], M. Stojanović[1], J. Milojković[1], V. Pantić[2], M. Mihajlović[1], M. Adamović[1]

[1]Institute for Technology of Nuclear and Other Mineral Raw Materials, Franchet d'Esperey 86, 11000 Beograd, Republic of Serbia
[2]Bio-Ecological Center d.o.o., Petra Drapšina 15, 23000 Zrenjanin, Republic of Serbia
Corresponding author: naukabec@bioec.rs
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Abstract: Assesment of the binding capacity of waste material – sour cherry stone particles (unmodified and modified), for different mycotoxins was tested *in vitro.* After proper preparation, two sorts of sour cherry shell particles were used: one as unmodified cherry shell particles (CS) and another one obtained by acid modification, marked as MCS. Adsorption of six mycotoxins: aflatoxin B1 (AFL), ochratoxin A (OTA), deoxynivalenol (DON), zearalenone (ZON), diacetoxyscirpenol (DAS) and T-2 toxin by CS and MCS was investigated *in vitro*. For adsorption experiments crude extracts of mycotoxins were used. The assesment of the ability for binding mycotoxins was evaluated in the electrolyte 0.1 M K_2HPO_4 which pH value was adjusted to 3.0 and 7.0, respectively. Mass ratio of individual mycotoxin and sour cherry shell samples was 1:5000. The experimental mixtures were incubated for 1 hour on a rotary shaker (185 rpm) at room temperature (22-25^o C). After incubation the extractions of nonadsorbed mycotoxins from the filtrates were performed with organic solvents, and their quantification was done by thin-layer chromatography. The amount of adsorbed AFL ranged from 41.18 (pH 7.0 - CS) to 58.82% (all other cases). Having in mind all analyzed mycotoxins, **i**t was noted that tested waste materials had the highest binding capacity for OTA at pH 3.0 (66.67% - CS and 76.20% - MCS). The quantity of adsorbed DON was similar by CS and MCS although it was somewhat higher by CS at pH 7.0 (30.00%). Binding of ZON was not depending of pH value of the applied electrolyte, but it was larger by MCS. DAS was adsorbed only by MCS at pH 7.0 while higher adsorption capacity for T-2 toxin by both sour cherry shell samples was recorded at pH 3.0.

Key words: mycotoxins, adsorption, *in vitro,* sour cherry stones, acid modification

Introduction

The aim of the present study was to evaluate and to compare *in vitro* the binding capacity of unmodified sour cherry stone particles with same particles that were treated with hydrochloric acid to six different mycotoxins: aflatoxin B1, ochratoxin A, deoxynivalenol, zearalenone, diacetoxyscirpenol and T-2 toxin. The technique used for the mycotoxins removing was biosorption, that have already proved to be efficient, low cost and sustainable technique that uses cheap and abundant biomaterials, usually declared as waste, for removing different pollutants. Among these pollutants the common are heavy metals and organic waste, mostly dies *(Naja et al., 2009; Sud et al., 2008; Das et al., 2008)*, but the recent investigatios have proved that similar waste material may be used for removing micotoxins as well *(Bočarov-Stančić et al., 2012a; 2012b; Lopičić et al., 2013a)*.

Many substances can contaminate the animal feedstuff, but the outstanding place among them belongs to mycotoxins. These naturally occurring components, secondary fungal metabolites, can have deleterious health effects to animals and humans, due to the consumption of contaminated animal products. One approach in finding method(s) for prevention of toxic effects of mycotoxins is detoxification and inactivation of these fungal metabolites by the use of mycotoxin binders. These additives inhibit the uptake of mycotoxins by animals *in vivo.*

Biosorption is technique that removes pollutants by different kind of cheap, abundant and easily available waste materials which can be divided in four main groups: living or non-living bacterial biomass, algae, fungi and agricultural waste materials *(Das et al., 2008)*. In this paper we have used agricultural waste material whose basic components are cellulose, hemicellulose, lignin, extractives, lipids, proteins, simple sugars, water hydrocarbons, starch etc. The presence of the three main biological polymers (cellulose, lignin, and hemicellulose) causes richness of cherry stone particles in hydroxyl and phenol groups which can be further chemically modified to produce adsorbent materials with improved adsorbing properties. Beside this, different agricultural waste materials also pose multilayer porous structure filled with openings and channels that provide huge volume per sorbent surface unit, which is favorable in biosorption process *(Hubbe et al., 2011; Lopicic, 2013b).*

There are many different types of modifications that can be used for improving adsorption capacities of biomaterials, such as physical, chemical, thermal or combined modifications Chemical modification of cellulosic materials is often used to improve certain properties of the material or some of its components, such as material hydrophlic or hydrophobic characteristics, its elasticity, adsorbtive or ion-exchange capability, thermal properties of the material or its resistance to microbiological attack, but in the most cases the chemical

modification serves to improve absorbing capacity of materials *(Sun, 2010)*. Many researchers have conducted their investigations using different chemical agents (acids, bases, etc.) in order to improve absorbing properties of biomaterials toward the heavy metals *(Wan Ngah and Hanafiah, 2008)*. Acid pretreatment serves for removal of some soluble organic impurities, to change the structure of functional cell compounds and to expose much more binding sites in order to improve biosorption capacity. In the case of heavy metals biosorption, acid pretreatment increased the overall negative charge of the adsorbents, which improved biosorption capacities for negative cations *(Elangovan et al., 2008)*. This kind of modification will also help in keeping pH value constant during the biosorption process, stabilizing the biosorbent and improving biosorption in the same time *(Allouche et al., 2011)*.

Material and Methods

Biosorbent preparation. Agricultural sour cherry stone waste material was obtained from "Vino Župa" Company from Aleksandrovac, where it has been disposed as by-product waste from their Juice Factory. After receiving, the sour cherry stone material was separated from the remainder of soft fruit residues, washed, dried at room temperature, and kept in polypropylene bottles for further treatment. Prior to experiment, the samples were manually crushed and separated from kernels, which were discharged, so for further analyses only hard stone part was taken. The crushed sour cherry stones were further milled to different fractions (in described experiment only the particles with diameter less than 100 μm were used), than washed several times in 0.01 M hydrochloric acid (HCl) in order to eliminate surface impurities, and then in distilled water until negative reaction with Cl^- ions. After that, the samples were dried at 60 ^oC for 24 h, and stored. One part of these sour cherry stone particles was directly used for mycotoxin adsorption as unmodified material (CS). Another sample marked as modified sour cherry stone particles (MCS) was activated by 1M HCl on thermostatic orbital shaker (25 ^oC and 200 rpm). After 1 hour, flask content was filtered, particles were washed with distilled water several times, and the procedure with 1M HCl was repeated two more times. At the end MCS was washed with distilled water until negative reaction with Cl^- ions. All the samples were marked and stored in polypropylene bags until the experiment started.

The chemical analyses of the samples concerning micro- and macroelements in unmodified sour cherry stones was performed according to standard chemical methods *(The Official Gazette of SFRY, No. 15/87)*. The content of biopolymers, cellulose, lignin as well as ADF and NDF was analyzed according to the method of Van Soest (*Van Soest et al., 1991)*.

Production, quantification and isolation of mycotoxins. Aflatoxin B_1 (AFL), ochratoxin A (OTA), deoxynivalenol (DON) and zearalenone (ZON) were produced by the solid substrate fermentations per the methods of *Bočarov-Stančić et al. (2009a, 2009b, 2010)*. Type A trichothecenes (diacetoxyscirpenol - DAS and T-2 toxin) were biosynthesized by submerged fermentation in liquid medium (*Bočarov Stančić et al., 2007)*. For the toxin production following fungal cultures used were: *Aspergillus flavus* GD-2 (leg. prof. dr G. Dimić, Technological Faculty, Novi Sad, Serbia), *A. ochraceus* CBS 108.08, *Fusarium graminearum* D2 (leg. dr A. Bočarov-Stančić, Bio-Ecological Center, d.o.o., Zrenjanin, Serbia), *F. semitectum* SL-B (leg. dr A. Bočarov-Stančić, Bio-Ecological Center, Zrenjanin, Serbia), and *F. sporotrichioides* ITM-391 (leg. dr A. Bottalico, Consiglio Nazionalle delle Richerce, Istituto Tossine a Micotossine da Parassiti Vegetali, Bari, Italy).

Isolations of mycotoxins and determinations of single mycotoxin content in solid substrates were done according to standard thin-layer chromatographic (TLC) method for fodder analysis *(The Official Gazette of SFRY, No. 15/87)*. Isolations of type A trichothecenes were done by ethyl acetate and their quantities were determined by TLC method according to *Rukmini and Bhat (1978)*. Isolated crude toxins were evaporated to dryness and dissolved in following solvents: ethanol (AFL, OTA, ZON), ethyl acetate (DAS, T-2) and methanol (DON). The final concentrations of stock mycotoxins' solutions were 0.1 µg/µl (AFL) and 1 µg/µl (OTA, DON, ZON, DAS and T-2), respectively.

Experimental procedure. In order to perform adsorption experiments stock solutions of mycotoxins were diluted as follows: AFL to 0.2 µg/ml, ZON to 0.8 µg/ml, and all other mycotoxins to 2.0 µg/ml with electrolyte (0.1M K_2HPO_4). The pH value of electrolyte was adjusted with 10% HCl or 20% NaOH to 3.0 and 7.0, respectively.

The binding ability of sour cherry stones was tested *in vitro* as follows: aliquots (50 ml) of test solutions were added to Erlenmayer flasks (250 ml) containing 500 mg of single biosorbent in the case of OTA, DON, DAS and T-2 toxin, 200 mg in the case of ZON, and 50 mg in the case of AFL. Controls were prepared by adding of 50 ml of the test solutions without adsorbent. The flasks were stoppered, incubated for 1 hour on rotary shaker (185 rpm) at room temperature (22-25^{o}C) and then filtered. In 25 ml aliquots of electrolyte with biosorbent (C) and without it (C_o) concentration's of mycotoxins were determined, after extraction with 2 x 15 ml of organic solvents: benzene (ZON), benzene-acetonitrile (AFL), and ethyl acetate (OTA, DON, DAS and T-2) respectively, by TLC methods *(The Official Gazette of SFRY, No. 15/87; Rukmini and Bhat, 1978)*. All analysis were performed in three replications and average values are presented.

The adsorption index of individual mycotoxin, in percentages, was calculated by the following formula:

$$Adsorption\ index = [\frac{C_0 - C}{C_0}]\ x\ 100$$

Results and Discussion

The chemical analyses of unmodified sour cherry stone particles were performed in order to elucidate the chemical composition and the content of micro- and macro- elements present in the materials. The obtained data are shown in Table 1 and Table 2.

As it can be seen from Table 1, sour cherry stone particles do not consist of elements which can be toxic to living organisms, so they can be used as additive for animal feed. From Table 2, where structural components of sour cherry stone particles are presented, it can be seen that sour cherry stone particle consist mostly of cellulose (54.74%) and lignin (17.47%).

Table 1. Micro- and macro-elements content in unmodified sour cherry stone particles (CS)

Parameter	CS (%)
Ca	0.52
P_2O_5	0.21
K	0.073
Na	0.040
Fe	0.02
SO_3	<0.02
Mg	0.017
Al	0.01
Cd	<0.01
Mn	<0.01
Pb	0.007
Cu	0.0021
Zn	0.0015
Ni	0.001
LOI *(600 ^oC)	99.56
LOI *(1000 ^oC)	99.71

Legend: *LOI=lost of ignition

Table 2. Chemical composition of unmodified sour cherry stone particles (CS)

Parameter (%)	CS (%)	DCS*(%)	JUS/ISO/ Documented methods (VDM)
Dry matter	94.09	100.00	Offic. Gazette of SFRY 15/87, Method 6
Crude cellulose	54.74	58.18	Method VDM-111
Nitrogen free extracts (NFE)	37.50	39.86	Offic. Gazette of SFRY 15/87, Method 20
Crude protein	1.48	1.57	Offic. Gazette of SFRY 15/87, Method 7
Ash	0.33	0.35	Offic. Gazette of SFRY 15/87, Method 18
Crude fat	0.04	0.04	Offic. Gazette of SFRY 15/87, Method 12
Fraction of fiber**			
Neutral detergent fiber (NDF)	65.18	69.27	Method VDM-118
Hemicellulose	2.71	2.88	Method VDM-118
Acid detergent fiber (ADF)	62.47	66.39	Method VDM-119
• Cellulose	45.00	47.82	Method VDM-119
• Lignin	17.47	18.57	Method VDM-119

Legend: *DCS-dry basis sour cherry stone composition
**NDF = ADF+ hemicellulose; ADF= Cellulose+lignin

The chemical composition as well as micro- and macro- element content in the sour cherry stone particles (Table 1 and Table 2) imply that this material can be used in animal feed as energetic material or even as carrier of certain active substances. Also, this waste material can be added as ingredient of complex mycotoxin binding additive for food and animal feed, that might have at the same time double effect: fungistatic and bacteriostatic, as it is described in patent No. 20120070516 (www.faqs.org/pattents/app/20120070516).

The adsorption indexes of six mycotoxins by unmodified biosorbent – sour cherry stone particles (CS) as well as sour cherry stone particles modified by acid (MCS), calculated on two pH levels, are given in Table 3.

Table 3. Adsorption indexes of six mycotoxins by modified and unmodified sour chery stone particles at different pH values

Adsorbent	pH	Adsorption index (%)					
		AFL	OTA	DON	ZON	DAS	T-2
Sour cherry stone particles (CS)	*3.0*	58.82	66.66	22.00	33.33	0	50.00
	7.0	41.18	20.00	30.00	33.33	0	40.00
Acid modified sour cherry stones particles (MCS)	*3.0*	58.82	76.20	22.00	50.00	0	50.00
	7.0	58.82	20.00	21.88	50.00	16.67	40.00

As it can be seen from results obtained by the use of TLC method (Table 3) unmodified sour cherry stone particles bound 58.82% of applied AFL at pH 3.0, while the increase from 41.18% to 58.82% with acid modification of the same waste material occurred at pH 7.0. The effect of pH value on OTA adsorption indexes was as follows: at pH level 3.0 increase from 66.66% (CS) to 76.20 % (MCS) was obtained, while no changes in binding of the same micotoxin occurred at pH 7.0. In the case of DON binding, it has been observed that adsorption indexes, by both analysed samples, remained constant at pH 3.0 (22.00%), while at pH 7.0 adsorption index decreased from 30.00% (CS) to 21.88% (MCS). Effect of electrolyte pH value on the binding of ZON was the same for both samples: particles of CS adsorbed 33.33% of ZON at both pH levels, while MCS adsorbed more of this mycotoxin (50.00%) at both pH values of K_2HPO_4 solution. In the case of type A trichothecenes (DAS and T-2 toxin) DAS was not bound on unmodified material at any pH value, while acid modification of sour cherry stone particles gave results only at pH 7.0 (16.67%). The amount of a bound T-2 toxin remained the same with and without modification: at pH 7.0 it was 40.00%, and at pH 3.0 the adsorption of 50% was recorded for this mycotoxin type.

Data presented in table 3 can be better observed by looking the graphs given in Figure 1 and Figure 2.

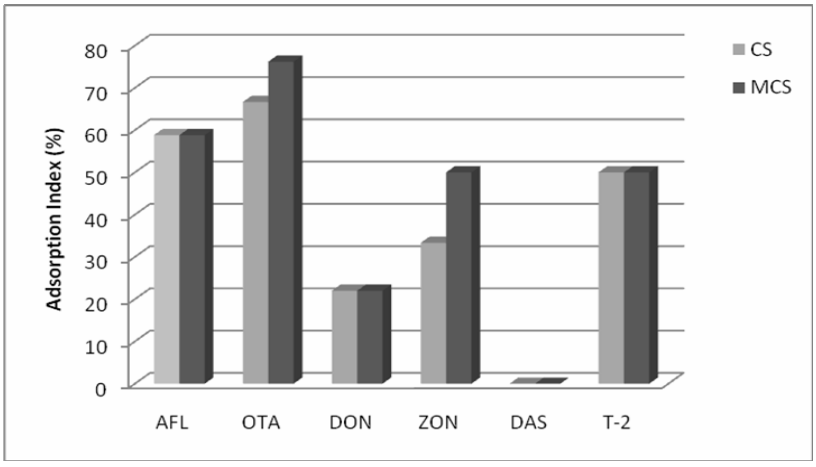

Figure 1. Comparison of adsorption indexes (%) for unmodified (CS) and modified sour cherry stone particles (MCS) at pH 3.0

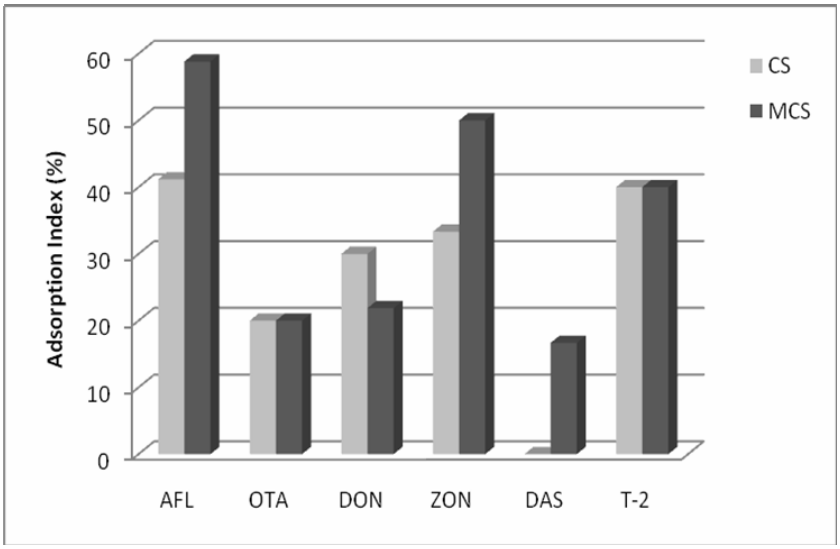

Figure 2. Comparison of adsorption indexes (%) for unmodified (CS) and modified sour cherry stone (MCS) particles at pH 7.0

As other food and agro-industrial waste cherry stones can be advantageous resource through thermal energy processes. Every year over 220 millions tons of food/agricultural waste are produced in Europe and recycling of these residues has received increasing attention mainly because of their environmental impact. One of the promising approaches is the use of lignocellulosic materials such as sour cherry stones as low-cost activated carbons *(Duran-Valle et al., 2005; Olivares-Marín et al., 2009)* or as copper biosorbents *(Lopičić et al., 2011; Lopičić et al., 2013b)*.

Generally, acid modification by 1M HCl has changed adsorption index for all six investigated mycotoxins, leading in most cases in improving of removal, except in the case of DON, where the adsorption index stayed constant at pH 3.0, while decrease from 30.00 % to 21.88 % at pH 7.0 occurred (Table 3). In the case of T-2 toxin, acid modification of sour cherry stone particles gave no change in adsorption index at both pH values. On the other hand, in the case of other four mycotoxins acid modification mostly improved the adsorption indexes, with the biggest increase in the case of ZON where the increase from 33.33% to 50.00% occurred at both pH values.

Similar results for biosorption capacity of another tipe of lignocellulose waste material were obtained in our previous study with unmodified and modified peach stones (*Lopičić et al., 2013)*. Acid modification with 1M HCl have changed the adsorption indexes for four, out of six tested mycotoxins (DON, ZON, DAS and T-2) leading to the improvement of their removal from experimental mixtures.

In the case of OTA adsorption indexes were the same for modified and unmodified material (peach stones) while in the case of AFL dicreased from 58.82 to 41.18 at both pH values was recorded.

The application of waste plant materials as ingredients of mycotoxin binding additives for food and animal feed has been described in US patent No. 20120070561 (www.faqs.org/pattents/app/20120070516). These additives are comprising 10-90% of modified ligno-polysaccharides and optionally 90-10% of conventional mycotoxin binding components, where the ligno-polysaccharide components are produced in some cases from food industry by-products such as cocoa shells, apricot stones, coffee grounds etc. For adsorption experiments *in vitro* used concentrations of mycotoxins (1 and 0.1 μg/ml, respectively) were much higher than naturally occurring one but were similar with the concentrations we have tested in our experiment (AFL - 0.2 μg/ml, ZON - 0.8 μg/ml, and all other mycotoxins - 2.0 μg/ml). Concentration of different plant adsorbents or their mixtures with conventional mycotoxin binding components was always the same – 5 mg/ml. According to the authors of the patent milled acid hydrolysed lignin bound 43.4% OTA, 23.3% DON, 98.1% ZON and 52.6% T-2 at pH 6.5. Lower adsoption indexes, obtained in our experiment (Figure 2) for OTA, ZON and T-2 may be the result of higher incubation temperature (37° C) and different content of micro- and macro-elements in compared biosorbents. According to the authors of the patent No. 20120070561, the ability for irreversible binding of different mycotoxins is based on the ability of porous lignocellosic materials to thermally collapse during melting and to entrap mycotoxins after closing lignin pore structure under high temperature treatment such as drying.

Conclusion

The results presented here indicate that the sour cherry stone particles can be used as effective biosorbents for mycotoxins *in vitro*. Acid modification leads to improving of biosorption capacity in most cases, but further investigations should be performed in order to elucidate the nature of interaction between the biosorbent and specific mycotoxin.

Like other *in vitro* asseys the presented assay cannot completely simulate the conditions in gastro-intestinal tract of animals, so further *in vivo* experiments are necessary to assess the efficacy of this waste lignoscelluosic materials as mycotoxins binders.

Acknowledgement

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In vitro adsorpcija mikotoksina košticama višnje

Z. Lopičić, A. Bočarov–Stančić, M. Stojanović, J. Milojković, V. Pantić, M. Mihajlović, M. Adamović

Rezime

U radu je prikazan *in vitro* model za evaluaciju sposobnosti adsorpcije različitih mikotoksina prirodnim otpadnim materijalom - košticama višnje, koje su dobijene iz kompanije "Vino Župa" iz Aleksandrovca. Koštice su odlagane kao otpad na deponiju nakon obrade voća i proizvodnje sokova. U Institutu za tehnologiju nuklearnih i drugih mineralnih sirovina, Beograd, izvršena je njihova obrada i pripremljena su dva uzorka, jedan nativan (CS), a drugi kiselinski aktiviran (MCS).

In vitro metodom testirana je adsorpcija šest mikotoksina: aflatoksina B1 (AFL), ohratoksina A (OTA), dezoksinivalenola (DON), zearalenona (ZON), diacetoksiscirpenola (DAS) i T-2 toksina. Za eksperimente adsorpcije su korišćeni sirovi ekstrakti mikotoksina, proizvedeni u Odeljenju mikrobiologije Bio-ekološkog centra d.o.o., Zrenjanin.

Sposobnost za *in vitro* vezivanje mikotoksina je ocenjivana u elektrolitu 0.1M K_2HPO_4 čija je pH vrednost podešena na 3,0 odnosno 7,0. Maseni odnos pojedinačnih mikotoksina i prirodnih mineralnih adsorbenasa je iznosio 1:5000. Eksperimentalne smeše su inkubirane tokom 1 sata na rotacionoj tresilici (185 o/min) i sobnoj temperaturi (22-25 ^oC). Nakon inkubacije vršena je ekstrakcija neadsorbovanih mikotoksina iz filtrata organskim rastvaračima i kvantifikacija istih metodom tankoslojne hromatografije.

Količina adsorbovanog AFL varirala je od 41.18 (pH 7.0 - CS) do 58.82% (u svim drugim slučajevima). Posmatrajući sve analizirane mikotoksine, primećeno je da je testirani otpadni materijal imao najveći adsorpcioni kapacitet u odnosu na OTA pri pH 3.0 (66.67% - CS i 76.20% - MCS). Količina adsorbovanog DON bila

je slična kod CS i MCS, iako su CS, pri pH 7.0, adsorbovale nešto više ovog mikotoksina (30.00%). Vezivanje ZON nije bilo uslovljeno pH vrednošću primenjenog elektrolita, ali je bilo nešto više u slučaju primene MCS. DAS su adsorbovale jedino MCS na pH vrednosti 7,0, dok je viši nivo adsorpcije T-2 toksina utvrđen na pH 3,0 kod oba ispitivana adsorbenta.

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DEVELOPMENT OF NIR CALIBRATION MODELS TO PREDICT CHEMICAL COMPOSITION AND *IN VITRO* ORGANIC MATTER DIGESTIBILITY OF MAIZE SILAGE

M. Maslovarić[1], B. de la Roza-Delgado[2], S. Janković[1], J. Lević[3], R. Jovanović[1]

[1]Institute for Science Application in Agriculture, Bulevar despota Stefana 68b, 11000, Belgrade, Republic of Serbia
[2]Regional Institute for Research and Agro-Food Development (SERIDA), E-33300, Villaviciosa (Asturias), Spain
[3]Institute of Food Technology – FINS, Bulevar cara Lazara 1, Novi Sad, 21000, Republic of Serbia
Correspondin author:
Marijana Maslovarić, e-mail: mmaslovaric@ipn.bg.ac.rs
Original scientific paper

Abstract: The aim of this research was to investigate the possibility of implementing Near Infrared Reflectance (NIR) spectroscopy method for determination of the chemical composition and *in vitro* organic matter digestibility of fresh, i.e. undried maize silage. The set of 142 fresh coarse silage samples silage was scanned in the reflectance mode (400–2500 nm) using Foss NIRSystem 6500 scanning monochromator. The same samples were analyzed by the reference laboratory methods in order to obtain calibration models for the content of dry matter (DM), crude protein (CP), ash, starch, fermentative parameters and neutral detergent fibre (NDF). The reference method for the analysis of organic matter digestibility was NDF - cellulase enzymatic method. Spectral data treatment and calibration model development were carried out using the WINISI 2 software v.1.05. Maize silage spectra were transformed by: Principal Component Analysis (PCA) regression, the Standard Normal Variate (SNV) plus Detrending (DT) methods. Mathematical treatments were applied in order to obtain spectra derivatives. The calibration models were developed using Modified Partial Least Squares regression technique (MPLS). The following parameters were used for the evaluation of the calibration models: the determination coefficient of calibration (R^2), standard error of calibration (SEC), standard error of cross validation, (SECV), determination coefficient of cross validation (1-VR) and residual predictive deviation (RPD) value. Calibration model for dry matter (DM) content was highly accurate and it can be used for analytical purposes, while models for

crude protein (CP), starch, fermentative parameters and NDF were less accurate and can be used for approximate assessment of the maize silage quality. Calibration models for ash content and *in vitro* organic matter digestibility of fresh, intact maize silage (IVOMD) were unsatisfactory, but they could be used to obtain rough predictions.

Key words: NIR, maize silage, chemical composition, in vitro digestibility

Introduction

Maize silage is an important forage crop in ruminant production systems (*Cozzolino et al., 2006*). It provides a low cost source of energy in the form of starch and fibre which complements pasture well for much of the year (*Kolver, 2001*). Quality of corn silages is commonly evaluated on the basis of its nutritive and fermentative characteristics. Chemical analysis of the maize silage provides data on the content of nutrients which is important for formulation of balanced rations for ruminants. However, knowledge of only the chemical composition in most cases is not sufficient parameter for assessing the nutritional value maize silage. For a more precise evaluation it is necessary to identify the degree of utilization of feed by animals. Therefore, the digestibility assessment as a criterion to assess the nutritional value of maize silage is considered to be extremely important in order to define its quality (*Jovanović, 2007*). Organic matter digestibility (OMD) is one of the indicators of the amount of available energy in feeds for ruminants (*Barber et al., 1990*). The most accurate method for determination of organic matter digestibility is by conducting *in vivo* experiments (*Pojić et al., 2008*). Also, various *in vitro* techniques have been developed, such as a method based on chemical composition (*Clancy and Wilson, 1966*), the gas production method (*Menke et al., 1979*), the nylon bag technique (*Mehrez and Orskov, 1977*), the rumen fluid *in vitro* procedure (*Tilley and Terry, 1963*) and an enzymatic *in vitro* technique (*De Boever et al., 1986*). Both chemical and digestibility analysis of maize silage are laborious, time consuming and expensive. Near infrared reflectance (NIR) spectroscopy is an alternative to traditional analytical methods, which comprises entire sample characterized in terms of its absorption properties in the NIR region and offers advantages of simplicity, speed of analysis and reduced chemical waste (*Murray, 1993; Deaville and Flinn, 2000; Stuth et al., 2003*). Both silage and forages have been analyzed routinely by NIR spectroscopy in dry and ground presentations to the spectrophotometer in many laboratories around the world (*Murray, 1993*). On the other hand, not many studies have been related to the analysis of fresh (undried)

maize silage samples by NIR spectroscopy. The use of fresh material might reduce preparation costs and possible compositional alterations during NIR analysis (*Cozzolino, 2006*). The aim of the present research was develop NIR calibrations for prediction of the chemical composition and *in vitro* organic matter digestibility (IVOMD) of undried maize silage samples.

Material and Methods

Silage samples (n=241) were obtained from the farms in Northern Spain during the 2008 - 2012 period. Fresh coarse samples were placed in a rectangular cell (internal dimensions of 4.7 cm wide, 20 cm long and 4.3 cm deep, with quartz window) and scanned in duplicate using Foss NIRSystem 6500 scanning monochromator (Foss NIRSystem, Silver Spring, MD, USA), with a scanning range of 400–2500 nm at 2 nm intervals. Spectra were recorded in the reflectance mode (log 1/R).

Spectral data treatment and calibration model development were performed using the WINISI 2 software v.1.05 (Infrasoft International Inc., Port Matilda, PA, USA). Principal Component Analysis (PCA) was performed on the set in order to decompose and compress the data matrix. After PCA, the centre of the spectral population was determined in order to detect outlier samples. The Mahalanobis distance (GH) was calculated between each sample and the centre; samples with an GH value greater than 3 were considered outliers (*Shenk and Westerhaus, 1996*). As spectral pretreatments, the Standard Normal Variate (SNV) plus Detrending (DT) (*Barnes, Dhanoa, and Lister, 1989*) procedure was used to remove the multiplicative interferences of scatter, and two derivative mathematical treatments were performed: window wise filtering (1,4,4,1) and (2,5,5,1) where the first digit is the order of the derivative, the second is the gap over which the derivative is calculated, the third is the number of data points in a running average or smoothing and the fourth is the second smoothing (ISI, 2000).The calibration models were developed using Modified Partial Least Squares regression technique (MPLS) included in WINISI software. Evaluation of the calibration models was carried out by internal cross validation. The following statistics were used for the evaluation of the calibration models: the determination coefficient of calibration (R^2), standard error of calibration (SEC), standard error of cross validation, (SECV), determination coefficient of cross validation (1-VR) and the other statistic used was residual predictive deviation (RPD) (*Williams and Sobering, 1996*), calculated as the ratio between the standard deviation of the reference data for the training set and the standard error of cross validation (SECV).

The same portion of each sample was analyzed by the reference laboratory methods in duplicate, after being oven dried at 60°C for 24 h and grounded in a

forage mill to pass a 0.75 mm screen. The content of the following quality parameters of maize silage were determined by chemical analysis: dry matter (DM), crude protein (CP), ash, starch, fermentative parameters (acetic, lactic and ammonia – N), neutral detergent fibre (NDF) and *in vitro* organic matter digestibility (IVOMD). The chemical analysis was performed using traditional analytical methodologies: dry matter content (DM) using dry - forced oven (60°C for 24 h) and corrected by residual DM at 103 C to constant weight; ash by incineration at 550 ºC to constant weight; crude protein (CP) by Kjeldahl analysis (*TECATOR, 1995*), neutral detergent fibre (NDF) using Van Soest and Robertson method (*Van Soest, Robertson and Lewis, 1991*); starch by using electrochemical procedure. Fermentative parameters were analysed in the juice obtained after pressing the maize silage samples: acetic and lactic acid by HPLC with ionic exchange and NH_3 N content by Kjeldahl destillation on the silage juice. All reference chemical analyses were performed in an accredited laboratory under the requirements of ISO/IEC 17025. *In vitro* organic matter digestibility (IVOMD) was determined by the NDF - cellulase enzymatic method (*Riveros and Argamentería, 1987*). The celulase enzyme used was Cellulase Onozuka R-10 (from *Trichoderma viride*). All results were expressed on as is basis.
Reference laboratory analysis and spectra collection were conducted at the Regional Institute for Research and Agro-Food Development (SERIDA), Asturias, Spain.

Results and Discussion

The variability of chemical composition and *in vitro* organic matter digestibility of the undried maize samples analysed in this research was considered suitable to develop NIR calibrations. Number of samples, mean, standard deviation, and the range of for the DM, CP, ash, starch, NDF contents, fermentative parameters content (acetic acid, lactic acid, ammonia – N) and IVOMD are given in Table 1.

Table 1. Descriptive statistics for the calibration set

Parameter	Mean	SD	Range
DM, %	32.33	3.87	24.42 - 50.53
Ash, %	1.15	0.24	0.48 - 2.44
CP, %	2.35	0.33	1.11 - 3.87
Starch, %	11.10	2.34	4.23 - 17.58
NDF, %	13.6	1.48	10.61 - 21.90
Acetic acid, mg/dl	858.3	431.31	52 - 2586
Lactic acid, mg/dl	2457	775	585 - 5787
NH3 N, mg/dl	32.4	11.36	6.5 - 92.3
IVOMD, %	69.25	3.03	57.85 - 76.83

SD: Standard deviation, DM: Dry Matter; CP: Crude Protein; NDF: Neutral Detergent Fibre; NH3 N: Ammonia; IVOMD: *In vitro* digestibility of organic matter

Evaluation of the predictive ability of the calibration models was performed by cross validation. Table 2 and Table 3 summaries the cross validation statistics for the measurement of chemical composition and fermentative parameters in maize silage samples by using two mathematical treatments – first (1, 4, 4, 1) and second (2, 5, 5, 1) order derivatives, respectively.

Table 2. NIR cross validation statistics for chemical composition and organic matter digestibility (OMD) in undried maize silage (mathematical treatment 1,4,4,1)

Parameter	Mean	SD	Range	T	SEC	R^2	SECV	1-VR	RPD
DM, %	32.54	3.46	24.42 - 42.68	6	0.67	0.94	0.83		
Ash, %	1.13	0.19	0.66 - 1.69	12	0.10	0.73	0.13	0.56	1.50
CP, %	2.34	0.26	1.60 - 3.00	12	0.09	0.88	0.11	0.81	2.28
Starch, %	12.30	2.14	5.60 - 16.51	8	0.80	0.83	0.90	0.83	2.38
NDF, %	10.88	3.62	5.68 - 18.45 -	13	1.21	0.89	1.50	0.81	2.42
Acetic acid, mg/dl	814.90	372.31	121.0 - 2071.5	13	105.95	0.92	137.72	0.86	2.70
Lactic acid, mg/dl	2688.23	678.32	945.2 - 4681.0	11		0.86	242.24	0.86	2.80
NH3 N, mg/dl	*	*	*	*	*	*	*	*	*
IVOMD, %	69.14	3.70	36.98 - 76.83	7	2.73	0.46	2.82	0.42	1.31

Table 3. NIR cross validation statistics for chemical composition and organic matter digestibility (OMD) in undried maize silage (mathematical treatment 2,5,5,1)

Parameter	Mean	SD	Range	T	SEC	R^2	SECV	1-VR	RPD
DM, %	32.12	3.44	24.42 - 42.68	6	0.68	0.96	0.80	0.95	4.28
Ash, %	1.14	0.20	0.66 - 1.79	5	0.12	0.65	0.15	0.49	1.39
CP, %	2.34	0.27	1.60 - 3.10	9	0.09	0.89	0.13	0.79	2.17
Starch, %	11.00	2.24	4.23 - 16.51	4	0.82	0.87	0.92	0.83	2.44
NDF, %	10.92	3.65	5.68 - 18.45	12	0.82	0.95	1.35	0.86	2.70
Acetic acid, mg/dl	816.40	378.46	121.0 - 2071.5	12	89.01	0.95	133.31	0.88	2.84
Lactic acid, mg/dl	2458.77	684.75	793.5 - 4681.0	8	190.80	0.92	241.24	0.88	2.84
NH3 N, mg/dl	31.98	10.24	8.46 - 58.60	8	3.34	0.89	4.55	0.80	2.25
IVOMD, %	69.29	2.96	57.85 - 76.83	3	1.69	0.67	1.80	0.63	1.65

SD: Standard deviation, DM: Dry Matter; CP: Crude Protein; NDF: Neutral Detergent Fibre; NH3 N: Ammonia; IVOMD: *In vitro* digestibility of organic matter; T: number of regression terms; R^2: determination coefficient of calibration; SEC: standard error of calibration; SECV: standard error of cross validation; 1-VR: determination coefficient of cross validation; RPD: residual predictive deviation value

When comparing the R^2, SECV, 1-VR, RPD values for these mathematical treatments, it can be observed that mathematical treatment using second order derivative (2, 5, 5, 1) provides calibration model with better prediction capability for all examined parameters. First order derivative (1, 4, 4, 1) gave higher R^2, RPD and 1-VR values, and lower SECV only for ash content, but the number of terms was very high (T=12), which indicates model overfitting. Therefore, further analysis was focused on the evaluation of the calibration models developed after mathematical treatment using second order derivative (2, 5, 5, 1).

The best accuracy of the NIR calibration model was obtained for DM content, which is consistent with the results of *Cozzolino et al. (2006)*. Cross validation statistics for DM content was as following: R^2 = 0.96, SECV = 0.80, 1-VR = 0.95 and RPD = 4.28. The R^2 was high, reaching value close to 1, while SECV was low. Particularly high value of RPD and high value of 1–VR confirm very good accuracy of the calibration model for DM content in maize silage.

Calibration model for the ash content in maize silage was characterized by the reduced values of cross validation statistics (R^2=0.65, SECV = 0.15, 1 – VR

=0.49, RPD = 1.39). This is could be explained by the fact that inorganic matter cannot be detected by near infrared electromagnetic radiation, therefore ash is measured indirectly, as the difference to organic matter.

Stuth et al. (2003) stated that good prediction accuracy is usually obtained when measuring protein content in feeds and forages (with R^2 of 0.95 or higher), which is related to strong (-N-H-) absorptions in the NIR region. However, in the present research, R^2 value for protein content in maize silage was below 0.90 (see Table 3). SECV was relatively low (0.13), while 1-VR (0.79) and RPD (2.17) values can be considered as intermediate. Intermediate RPD value for protein content calibration model could be explained with the fact that protein content in maize silage is low, so -N – H- absorption is not so significant, compared to high protein feedstuffs. Furthermore, errors in the reference method may occur.

Since starch is the major energy source in maize silage, NIR measurement accuracy of starch content is quite important. Cross validation statistics for starch content in maize silage was as following: R^2 = 0.87, SECV = 0.92, 1-VR = 0.83, and RPD = 2.44. Moreover, the number of MPLS terms used to develop the calibration model for starch was low (T = 4), thus, it may be concluded that the calibration model for starch obtained in this research had fair prediction capability.

According to the cross validation statistics given in Table 3, calibration model developed for NDF content in maize silage, has shown good predictive capability. High R^2 (0.95), 1-VR (0.86) and RPD (2.70) values were obtained. SECV was high, but due to the wide spread of NDF content (high SD), RPD value was very good.

Research of *Sorensen et al. (2004)* has shown that fermentative parameters of maize silage - lactic acid, and NH_3 N concentrations could be determined with a better accuracy when NIR measurements are performed on dried material rather than on wet material. In the same research acetic acid concentration was determined slightly more accurately when NIR measurements were performed on wet material because acetic acid is partially evaporated during the drying process, which could indicate that the calibration does not measure acetic acid directly but merely measures properties correlated with acetic acid (*Sorensen et al., 2004)*. On the other hand, *Park et al. (2006)* reported very good calibration models for acetic and lactic acid, when NIR measurement was carried out on undried maize samples. In the present research, cross validation statistics showed very good prediction potential of the calibration models for lactic and acetic acid. High R^2, 1 – VR, RPD values were obtained for both acetic and lactic acid (see Table 3).

Calibration model for NH3 N content in maize silage obtained in this research was less accurate than calibration models for lactic and acetic acid. According to the cross validation parameters (R^2 = 0.89, SECV = 4.55, 1-VR =

0.80, RPD = 2.25), we can conclude that this calibration model had intermediate prediction capability.

According to the cross validation statistics for the *in vitro* organic matter digestibility (IVOMD), it can be concluded that the prediction capability of the calibration model obtained for this parameter was unsatisfactory. Low predictive capability of the calibration model for measuring the *in vitro* organic matter digestibility (IVOMD) in undried maize silage was reported by *Cozzolino et al. (2006)*. These results could be explained by the fact that errors that may occur in the reference method, which is complex and tedious, and includes number of steps, increases the uncertainty and the errors in the calibration model. Furthermore, high moisture content of the samples has a significant effect on the accuracy of the NIR calibration models, since water absorbs energy in the NIR region. Inhomogeneity of the undried samples might also have a negative effect on the accuracy of the NIR calibrations (*Cozzolino et al. 2006*).

Conclusion

The results of this research showed that NIR spectroscopy can be useful in the prediction of dry matter (DM), crude protein (CP), starch, fermentative parameters (acetic, lactic and ammonia – N) and neutral detergent fibre (NDF) in undried maize silage. Calibration model for dry matter (DM) content can be used for analytical purposes, while models for crude protein (CP), starch, fermentative parameters (acetic, lactic and ammonia – N) and neutral detergent fibre (NDF) were less accurate and can be used for approximate assessment of the maize silage quality. Calibration models for ash content and *in vitro* organic matter digestibility (IVOMD) enabled values to be classified as either high or low, and they could be used to obtain rough predictions.

Fast determination of the chemical composition and organic matter digestibility of maize silage using the NIR method is very important for proper formulation of animal rations. Since organic matter digestibility is especially important quality parameter, even less accurate calibration models could be used for ranking maize hybrids in plant breeding programmes according to their suitability for preparing silage. Nevertheless, further research is necessary in order to be able to predict this parameter more accurately.

Using dried and milled samples of maize silage for NIR measurements could improve calibration models accuracy for maize silage quality parameters, but it is also costly and time – consuming.

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Razvoj NIR kalibracionih modela za određivanje hemijskog sastava i *in vitro* svarljivosti organske materije silaže kukuruza

M. Maslovarić, B. de la Roza-Delgado, S. Janković, J. Lević, R. Jovanović

Rezime

Cilj ovog rada bio je da se ispita mogućnost primene NIR metode za određivanje hemijskog sastava i *in vitro* svarljivosti organske materije u uzorcima silaže kukuruza bez njihove prethodne pripreme, u smislu sušenja i mlevenja. U tu svrhu, 241 uzorak silaže skeniran je na Foss NIRSystem 6500 uređaju, metodom refleksije (spektralni oseg: 400 – 2500 nm). Isti uzorci su analizirani referentnim laboratorijskim metodama u cilju dobijanja kalibracionih modela za sadržaj suve materije (DM), sirovih proteina, pepela, skroba i neutralnih deterdžentskih vlakana (NDF). Referentna metoda za analizu svarljivosti organske materije bila je *in vitro* NDF - celulaza enzimska metoda.

Za obradu spektralnih podataka primenjene su: analiza glavnih komponenata (PCA), standardna normalna varijabla i detrend (SNV - DT) i derivatizacija (izvod spektra). Kalibracioni modeli razvijeni su uz pomoć parcijalne regresije najmanjih kvadrata (Modified Partial Least Squares – MPLS). Obrada spektralnih podataka i razvoj kalibracionih modela izvedeni su u WINISI 2 softveru, v.1.05. Za evaluaciju kalibracionih modela korišćeni su parametri: koeficijent determinacije (R^2), standardna greška kalibracije (SEC), standardna greška unakrsne validacije (SECV), koeficijent determinacije unakrsne validacije (1-VR) i vrednost predikcije rezidulane devijacije (RPD).

Kalibracioni model za sadržaj suve materije ima visoku tačnost i može se koristiti za određivanje sadržaja suve materije pomoću NIR metode u analitičke

svrhe. Kalibracione modele za sadržaj sirovih proteina, skroba, fermentacionih parametera, i neutralnih deterdžentskih vlakana (NDF) karakterisala je manja tačnost, i oni se mogu koristiti za dobijanje orijentacionih vrednosti ovih parametara u silaži kukuruza. Kalibracioni modeli za sadržaj pepela i *in vitro* svarljivosti organske materije pokazali su nezadovoljavajuću tačnost, ali bi se i oni, mogli koristiti za okvirnu predikciju ovih parametara u uzorcima kukuruzne silaže.

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THE EFFECT OF CUTTING SCHEDULE ON ENERGY VALUE OF FIRST ALFALFA GROWTH

D. Terzić, B. Dinić, R. Stanisavljević, D. Đokić, J. Marković, J. Milenković, S. Anđelković

[1]Institute of Forage Crops, Kruševac 37251, Serbia
Corresponding author: dragan.terzic@ikbks.com
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Abstract: The influence of time of cutting of the first alfalfa growth on content and yield of net energy was investigated in the present study. The objective of the study was to determine the content and net energy yield in the first cut of alfalfa in a model of dual exploitation (production of roughage and seed production). Cutting of the first growth took place early (around 5th of May), medium early (about 15th of May), late (about 25th of May) and very late (around 5th of June). The second growth was used for seed production. In addition, we investigated two pre-cuts where cutting of the first growth was performed in the period around 5th of May, second around 5th of June, and the third growth was used in seed production.The cutting exerted a great influence on the net energy value (NE_L) of pre-cut. Results indicated a decrease in net energy value of alfalfa in the later stages of the exploitation of the first cut. The highest value of NE_L in the system of one pre-cut was realized in the early A_1 cutting system (5.40 $MJkg^{-1}DM$) and the lowest average value of NE_L of 3.88 $MJkg^{-1}DM$ was observed at very late time cutting (A_4). The net energy value realized in meat production (NE_M) showed similar trends as the net energy value of milk production. In the early (A_1) system value of 5.28 $MJkg^{-1}DM$ was achieved and the lowest value was recorded in very late (A_4) the system (3.41 $MJkg^{-1}DM$). The highest average production of NE_L and NE_M was realized in a system with two pre-cuts A_5 (31981 $MJha^{-1}$ and 31163 $MJha^{-1}$). In the variant with one pre-cut, the highest production of NE_L and NE_M was formed in the early (A_1) system (20988 and 20508 $MJha^{-1}$). Later cutting of the first growth resulted in decrease of production of NE_L and NE_M and the lowest value was recorded in very late (A_4) system of cutting (18199 and 16017 $MJha^{-1}$).

Key words: alfalfa, yield, first cut, net energy

Introduction

Alfalfa (*Medicago sativa L.*) is the most important perennial forage plant in Serbia. The significance of production of alfalfa is reflected in the production of high-value roughage, but the alfalfa seed is also valuable commodity in domestic and export markets. Alfalfa seed in Serbia is produced in the system of double exploitation (forage-seed). Seed is usually produced from the second growth, and the optimal pre-cut harvest period depends on seed yield and quality but also onyield and quality of forage. Quality of alfalfa dry matter is determined by growing conditions, genotype and stage of development especially at the time of cutting. Cutting alfalfa in the later stages of development provides greater yield and durability of alfalfa (*Lioveras, 2001, Undersander et al., 2004*). The best quality and the highest yield of nutrients is in the early stages of growth of alfalfa - in the early stages of budding and flowering (*Bošnjak et al., 1983; Mejakić et al., 1997). Terzić (2010)* and *Terzić et al. (2012)* indicate that postponing of cutting from the early to the late period results in the protein content decrease and increase of the yield of dry matter, while the crude protein yield per unit area remains even.

The cutting schedule and number of pre-cuts significantly affect alfalfa seed yield (*Karagić et al., 2006, Terzić 2010*). *Karagić et al., 2006* and *Terzić 2010* have established that later first cut in average results in the increase of the seed yield from the second cut compared to the yield of pre-cut in early May.

Notwithstanding the importance of seed yield and quality in this model of production it is important to determine the yield and quality of roughage produced in order to fully perceive the optimal time of cutting and fodder produced be better adapted to the needs of animals.

The aim of this study was to determine the average content and net energy yield of alfalfa dry matter in different systems of cutting using the dual exploitation model.

Material and methods

Study was carried out on experimental field of the Institute for forage crops in Kruševac. Trial was established in 2002, and the results obtained in 2005, 2006 and 2007 are presented in the present paper. Size of the basic parcel was 10.5 m^2. Factors were different time of pre-cut of alfalfa seed crop: A_1- early cut (around May 5th), A_2- medium early cut (around May 15th), A_3- late cut (around may 25th), A_4- very late cut (around June 5th), A_5- cut (A5 I- around May 5^{th} and A5 II - around June 5^{th}). In regard to levels of factors A_1, A_2, A_3, A_4, the second cut is used as seed cut, whereas in case of treatment A_5 the third cut was used as seed cut.

Cutting of growth for forage was done manually at height of about 5 cm. All results related to chemical composition are expressed through absolute dry matter. The quantity of nutrients was calculated based on the chemical composition and yield in absolute dry matter. Chemical analyses were carried out in the laboratory of the Institute for forage crops in Kruševac. The NE_L and NE_M values were calculated ($NE_L=k_1$ x ME, q=ME/UE x 100; $NE_M=k_{mf}$ x ME, q= ME/UE x 100). The calculation used the following digestibility coefficients (*Obračević 1990*).

Table 1 Digestibility coefficients in % (*Obračević 1990*)

Development stage	CP	CF	DM	NFE
$A_1$and A_5first cut (before flowering)	74	52	44	73
A_2 (beginning of flowering)	69	48	39	67
A_3 (full flowering)	66	43	34	63
A_4 (post-flower)	62	42	32	57
Second cut (before flowering)	80	53	46	78

Statistical processing of obtained data was done by variance analysis. Testing of the significance of differences was done by LSD test

Results and discussion

The energy value of alfalfa pre-cut depending on the cutting system is shown in the following table.

Table 2. $NE_L$and NE_M content depending on the cutting schedule ($MJkg^{-1}DM$)

	Year						Average content	
	2005		2006		2007			
Cutting schedule	NE_L	NE_M	NE_L	NE_M	NE_L	NE_M	NE_L	NE_M
A_1	5.37	5.25	5.47	5.34	5.37	5.24	5.40	5.28
A_2	4.71	4.43	4.81	4.56	4.74	4.50	4.76	4.50
A_3	4.22	3.82	4.30	3.94	4.33	4.00	4.29	3.92
A_4	3.85	3.37	3.94	3.50	3.84	3.36	3.88	3.41
A_5 (I)	5.41	5.34	5.45	5.32	5.38	5.31	5.41	5.32
A_5 (II)	5.71	5.50	5.78	5.51	5.72	5.50	5.73	5.50

(I)- first pre-cut (II) –second pre-cut

The results in all the years indicate a decrease in net energy value of alfalfa in the later stages of exploitation. In average the highest value was recorded in the second pre-cut of the A_5 system of cutting (5.73 MJkg^{-1}DM). In the system with a single pre-cut, the highest value was recorded in early (A_1) the system (5.40 MJkg^{-1}DM). By delaying the first cut, the net energy in milk production decreased. The lowest average value of NE_L of 3.88 MJkg^{-1}DM was observed at very late cutting time (A_4).

The average realized net energy value of meat production (NE_M) showed similar trends as the net energy value of milk production. In pre-cuts in all the years the highest NE_M value was recorded in the second cut in the A_5 system of cutting (5.5 MJkg^{-1}DM). In the early (A_1) system, an average of 5.28 MJkg^{-1}DM was achieved. Later cutting resulted in decrease of these values and the lowest value was recorded in very late (A_4) the system (3.41 MJkg^{-1}DM).

Reduction of the energy value of alfalfa in the later stages of exploitation is consistent with the results *Obračević (1990), Dinić (1997), Đorđević et al. (2003).*

The obtained values of NE_L and NE_M were at the level reported by *Dinić (1997)* where the average value of NE_L and NE_M in alfalfa silage was 5.25 MJkg^{-1} and 5.34 MJkg^{-1}DM in budding stage, while lower values were determined in the start of flowering (5.20 MJ kg^{-1} DM and 5.12 MJ kg^{-1} DM). Similar values have been stated by *Obračević (1990)* for alfalfa naturally dried, values for NE_L and NE_M ranging from 4.86 and 4.64 for the first cut in the pre-flowering stage, to 4.14 and 3.72 for the first cut of post-flower alfalfa. The same author have stated the higher energy values of dehydrated alfalfa-where the value of NE_L and NE_M ranged between 5.50 and 5.50 in the bud stage to 4.86 and 4.64 at the start of flowering. Slightly lower values of alfalfa hay have been observed by *Đorđević et al. (2003)* pre-flowering NE_L and NE_M values were 4.32 and 4.19 MJkg^{-1}DM, at the flowering stage 3.93 and 3.70, after flowering 3.59 and3.28, and in coarse alfalfa 3.40 and 3.04 MJkg^{-1}DM.

The amount of net energy per unit area is shown in Table 3. The influence of cutting system on the amount of NE_L in pre-cuts is very important. The highest production of NE_L from pre-cuts in all three years in the system of cutting with two pre-cuts was obtained in A_5 system (31981 MJha^{-1}), which is by 52.4% more compared to the A_1 system where production was formed (20988 MJha^{-1}) in the variant with a single pre-cut. In all the years, in the first cut in the A_5 system, lower production was formed compared to the A_1 system. Also, the second cut has achieved the lowest average production of NE_L (12704 MJha^{-1}), however, the total production of two pre-cuts contributed to the formation of the highest productions in the A_5 system of cutting. Delay in cutting of the first growth contributed to the reduction of the energy value from the first to the fourth cutting schedule. The A_4

system of cutting resulted in the lowest production (18199 MJha^{-1}) or by 13.3% less compared to the A_1 system.

Table 3. Quantity of NE_L and NE_M in pre-cuts, MJ ha^{-1}

Cutting schedule	Year						Average content	
	2005		2006		2007			
	NE_L	NE_M	NE_L	NE_M	NE_L	NE_M	NE_L	NE_M
A_1	22427	21915	20235	19772	20301	19838	20988	20508
A_2	21259	20000	19857	18827	18984	17999	20033	18942
A_3	20550	18600	18665	17070	18612	17196	19276	17622
A_4	19753	17307	18686	16587	16159	14158	18199	16017
A_5 (I)	21287	20989	18730	18302	17813	17563	19277	18951
A_5 (II)	9343	8995	10847	10383	17923	17256	12704	12211
A_5 (I +II)	30630	29984	29577	28685	35736	34819	31981	31163
F- test	**	**	**	**	**	**	Level	
LSD	941	875	925	884	1301	1241	5%	
	1369	1274	1228	1171	1892	1806	1%	

Analysis of the three-year production of NE_M from pre-cuts indicates that in a system with single pre-cut, in all the years, the highest production was formed in the A_1 cutting schedule, in average of 20508 MJha^{-1}. In the A_2 and A_3 cutting schedules, lower production in average by 7.6% and 14.1% was realized, compared to the early A_1 schedule. The lowest production was formed within the A_4 cutting schedule (16017 MJha^{-1}), which is by 21.9% less than the production within A_1 schedule. The two cuts in the A_5 system have contributed to the formation of the highest productions in this system of cutting (31163 MJha^{-1}).

Conclusion

The cutting schedule exerted a great influence on the net energy value of biomass of alfalfa pre-cuts. Results indicate a decrease in net energy value of alfalfa in the later stages of the exploitation of the first cut.

In the early (A_1) system, the highest values of the NE_L and NE_M (5.40 and 5.28 MJkg^{-1}DM) were achieved in a system with a single pre-cut. Delay in cutting of the first growth contributed to the reduction of the energy value. The lowest average values of NE_L and NE_M of 3.88 and 3.41 MJkg^{-1}DM were observed at very late cutting schedule (A_4).

The highest average production of NE_L and NE_M was realized in a cutting system with two pre-cuts A_5 (31.981 $MJha^{-1}$ and 31.163 $MJha^{-1}$). In the variant with one pre-cut, the highest yield was formed in the early (A_1) system (20988 and 20508 $MJha^{-1}$). Later cutting of the first cut resulted in the decreased production of NE_L and NE_M and the lowest value was recorded in very late (A_4) system of cutting (18199 and 16017 $MJha^{-1}$).

In all the years of study, in pre-cuts, higher energy value of milk production was realized compared to the net energy value of meat production.

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Uticaj vremena kosidbe na energetsku vrednost prvog porasta lucerke

D. Terzić, B. Dinić, R. Stanisavljević, D. Đokić, J. Marković, J. Milenkvić, S. Anđelković

Rezime

U radu je ispitivan uticaj vremena kosidbe prvog porasta lucerke na sadržaj i prinos neto enrgije. Cilj rada je bio da se u modelu dvojakog iskorišćavanja (proizvodnja voluminozne hrane i proizvodnja semena) utvrdi sadržaj i prinos neto energije u prvom porastu lucerke. Kosidba prvog porasta je obavljena je rano (oko 5 maja), srednje rano (oko 15 maja), kasno (oko 25 maja) i veoma kasno (oko 5 juna). Drugi porast korišćen je za proizvodnju semena. Pored toga ispitivani su i dva predotkosa gde je kosidba prvog porasta obavljena u period oko 5 maja, drugog oko 5 juna, a treći porast za proizvodnju semena.

Vreme kosidbe je ispoljilo snažan uticaj na neto energetsku vrednost (NE_L) predotkosa. Rezultati ukazuju na smanjenje neto energetske vrednosti lucerke u kasnijim fazama iskorišćavanja prvog otkosa. Najveća vrednost NE_L-a u sistemu sa jednim predotkosom je ostvarena u ranom A_1 sistemu kosidbe (5,40 $MJkg^{-1}SM$) a najmanja prosečna vrednost NE_L-a od 3,88 $MJkg^{-1}SM$je dobijena u veoma kasnom roku kosidbe (A_4). Ostvarena neto energetska vrednost u proizvodnji mesa (NE_M) je pokazala slične tendencije kao i neto energetska vrednost u proizvodnji mleka. U ranom (A_1) sistemu je ostvareno 5,28$MJkg^{-1}SM$, a najmanja vrednost je ostvarena u veoma kasnom (A_4) sistemu (3,41 $MJkg^{-1}SM$).

Najveća prosečna produkcija NE_L-a i NE_M-a je ostvarena u sistemu kosidbe sa dva predotkosa A_5 (31981 $MJha^{-1}$ i 31163 $MJha^{-1}$). U varijanti sa jednim predotkosom najveća produkcija NE_L-a i NE_M-a je formirana u ranom (A_1) sistemu (20988 i 20508 $MJha^{-1}$). Kasnijom kosidbom prvog otkosa produkcija NE_L-a i NE_M-a opada i najmanja je u veoma kasnom (A_4) sistemu kosidbe (18199 i 16017 $MJha^{-1}$)

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EFFECT OF N FERTILIZATION ON YIELD AND QUALITY OF GRASSES AND LEGUMES IN MONOCULTURE AND MIXTURE

Z. Tomić[1], Z. Bijelić[1], V. Mandić[1], A. Simić[2], V. Caro-Petrović[1], M. Žujović[1]

[1]Institute for Animal Husbandry, 11080, Belgrade-Zemun, Republic of Serbia
[2]Faculty of Agriculture, Zemun-Belgrade, Republic of Serbia
Corresponding author: zonesh@gmail.com
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Abstract: The experiment was conducted on the experimental field of the Institute for Animal Husbandry in the period 2010-2012. Investigations included pure crops of alfalfa (A1), cocksfoot (A2), meadow fescue (A3) and English ryegrass (A4) and their mixtures in different combinations (50% alfalfa and cocksfoot - A5, 50% alfalfa, 25% cocksfoot and 25% meadow fescue - A6, 25% alfalfa, 50% cocksfoot and 25% meadow fescue - A7 and 40% alfalfa, 20% cocksfoot, 20% meadow fescue and 20% English ryegrass - A8). N fertilization was carried out early in the spring, with the movement of the vegetation in the three treatments: 0 (B1), 50 (B2), and 100 kg ha^{-1} (B3). The aim of this study was to investigate the effect of different amounts of N fertilizer on production, quality and energy characteristics of forage crops as monoculture and mixture. Crop as tested factor had a statistically significant effect on dry matter yield and contents of CP, NFE, NDF, ADF, ME and NEL and N fertilization on yield and NFE and the NDF content.

Key words: grass, legume, mixture, N fertilization

Introduction

Fodder produced by sowing of pure cultures of grasses and legumes or mixtures thereof, is very important in the field of farming of animals at all levels of production intensity. The organization of production should be such as to provide a sufficient amount of high quality forage for proper feeding of animals throughout the year. Because of the many advantages of mixtures of grasses and legumes in relation to their individual growing, such as uniformity of yield during the growing season, reduced need for nitrogen fertilizers, less weed, greater balance of meal for

animals, they should introduced into production as much as possible in lowland as well as in mountainous conditions. According to data obtained by *Chakarov and Vassilev (1992)*, alfalfa mixtures with grasses, cocksfoot and tall fescue, gave higher yields compared to pure alfalfa crop by 22.3 and 14.5%. According to the results obtained by *Nešić (2006),* depending on the compatibility of the species in the mixture and their reaction to the amount of N fertilizer, yields of mixtures can reach yields of pure alfalfa crop. Thus alfalfa in pure crop yielded 15.91 t ha^{-1} and in mixtures with cocksfoot and tall fescue 15.47 t ha^{-1}. Adding N fertilizer leads to an increase in dry matter yield and crude protein content. Alfalfa without fertilization achieved yield by 8.7% higher than the yield of its mixtures with cocksfoot and tall fescue, and in fertilization treatment with 210 kgN ha^{-1} yield of mixtures was higher for 7.84% (*Nešić et al., 2007*). According to *Tomić et al. (2012),* crude protein content has increased by fertilization from 9.90 to 13.10%. What is very important for livestock production is the energy value of the feed. It largely depends on the stage of maturity of the crop. Metabolizable energy content decreases with aging of crops. This reduction is lower in grass-legume mixtures than in pure grass crops (*Lättemäe and Tamm, 1997*).

The aim of this study was to investigate the effect of different amounts of N fertilizer on production, quality and energy characteristics of forage crops as monoculture and mixture.

Material and Methods

The experiment was conducted on the experimental field of the Institute for Animal Husbandry according to the plan of randomized block design with four replications. Sowing within the experiment was conducted in the fall of 2010. Sowing included pure crops: alfalfa (A1), cocksfoot (A2), meadow fescue (A3) and English ryegrass (A4), and their mixtures of 50% alfalfa and cocksfoot (A5), 50% alfalfa, 25% cocksfoot and 25% meadow fescue (A6), 25% alfalfa, 50% cocksfoot and 25% meadow fescue (A7) and 40% alfalfa, 20% cocksfoot, 20% meadow fescue and 20% English ryegrass (A8) (Table 1). The effect of three levels of nitrogen (B1 = 0, B2 = B3 = 50 and 100 kg ha^{-1}) on yield, quality and energy content of dry matter of the grassland, pure cultures and their mixtures was also investigated. Nitrogen was applied in the form of KAN in the spring, at the beginning of vegetation. Analysis of forage quality was done by standard laboratory methods in the laboratory of the Institute for Animal Husbandry, and the energy values of feedstuffs were calculatedm by using the following formulas:

1. Metabolizable Energy (ME), (*NRC,* 1989):
   ME (Mcal/kg) = 1.01 x DE (Mcal/kg) - 0.45
   DE (digestible energy)

DE (Mcal/kg) = 0.04409 x TDN(%)
TDN (Total digestible nutrient)
TDN(%)= 88.9-(ADF x 0.779)

2. Net energy for lactation (NEL), (*NRC*, 1989):
NEL (Mcal/kg) = 0.0245 x TDN(%) -0.12

Table 1. Sowing norm

Species, cultivar	Sowing norm kg ha^{-1}
Alfalfa, K-28	20.0
Cocksfoot, Baraula	50.0
Tall fescue, K-21	50.0
English ryegrass, Esquire	50.0

In the fall, for thepurpose of preparation of the soil for sowing, superphosphate was applied at a rate of 165 kg ha^{-1} of pure phosphorus. The required amount of phosphorus fertilizer was determined based on the content of available phosphorus in the soil and crops requirements (*Kresović, 2010*).

Main agrochemical properties of the soil on which the trial was performed are shown in Table 2.

Table 2. Main agrochemical properties of the soil

Depth cm	pH	Humus	Total N	C/N	Available N mg kg^{-1}			mg $100g^{-1}$	
	H_2O	%			NH_4-N	NO_3-N	Sum	P_2O_5	K_2O
0 – 30	6.2	0.19	3.31	10	23.1	23.8	46.9	5.4	18.4

Based on the obtained results of soil pH, it can be concluded that the soil on which the experiment is performed, belonged to the group of acid soils. According to humus content, soil had medium humus content and medium total nitrogen content. The ratio C/N is typical of arable land. According to the determined easy available phosphorus content, this soil is classified as a poor, and in regard to the content of easy available potassium this soil is categorized in the group medium provided soils. The content of ammonium and nitrate nitrogen was balanced and the total content of available nitrogen was 46.9 mg kg^{-1}.

The following table shows the results of the average monthly air temperatures and total precipitation.

Table 3. Mean air temperatures (°C) and total precipitation (mm) in 2012

Months	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	x / Σ
Temperature	2.1	-3.0	10.1	14.5	17.9	24.6	26.9	26.2	21.5	14.6	10.5	2.0	14.0
Precipitation	87.4	61.5	2.4	66.9	127.9	16.0	39.0	4.5	29.4	44.9	29.2	54.6	563.7
Temperature (1981-2010)	1.4	3.1	7.6	12.9	18.1	21.0	23.0	22.7	18.0	12.9	7.1	2.7	12.5
Precipitation (1981-2010)	46.9	40.0	49.3	56.1	58.0	101.2	63.0	58.3	55.3	50.2	55.1	57.4	690.9

The values of mean monthly temperatures and total precipitation deviated significantly from the long-term average values, which had adverse impact on crop production. Temperatures were, on an annual basis, higher than long-term average by 1.5 ° C, and the precipitation lower by 127.2 mm. Distribution of rainfall during the growing season was also negative causing an adverse occurrence of longer periods of drought that have reduced yield of crops tested in all cuts and significantly reduced the amount of total dry matter yield and quality of forage plants (Figure 1).

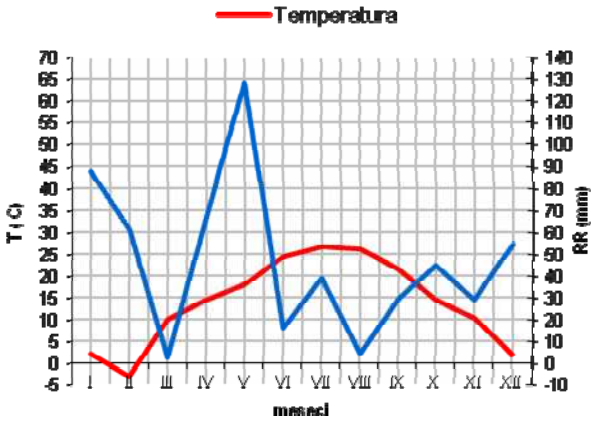

Figure 1. Climate diagram according to Walter for year 2012.

Results and Discussion

The total dry matter yield significantly depending on the type of grassland. Pure alfalfa crop(A1) achieved significantly higher yields compared to grass crops and mixtures of alfalfa with grasses. Yields of mixtures were significantly higher than the yields of pure grass crops. The highest dry matter yield was achieved by A6 mixture of 5.87 t ha^{-1} and minimum A7 mixture of 4.74 t ha^{-1}. Cocksfoot in

monoculture achieved the yields that were not statistically different from the yields of mixtures A7 and A8. English ryegrass had the lowest yield of 2.44 t ha^{-1} (Figure 2).

With the increase of the amount of nitrogen also dry matter yield increased (Figure 3). Nitrogen application in early spring stimulated the growth of grasses and legumes. The variant B3 (100 kg N ha-1) yield was significantly higher by1.59 t ha^{-1} compared to control and by 0.78 t ha^{-1} compared to the B2 variant with 50 kg N ha^{-1}. In the variant B2 yields of 4.91 t ha^{-1} were achieved, which were also significantly higher than the yield in the control variants.

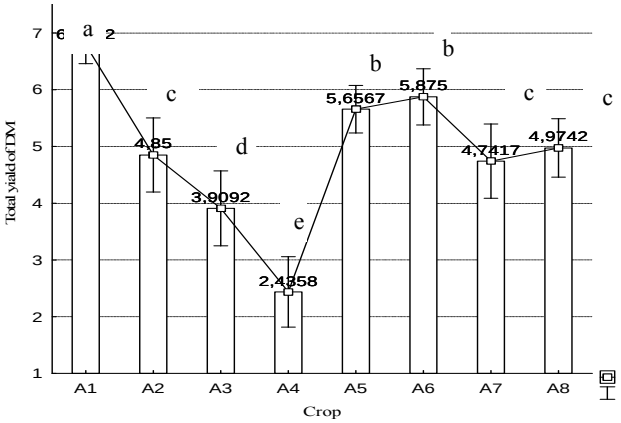

Figure 2. Influence of the type of crop on the total dry matter yield t ha^{-1}

In the research by *Balan et al. (2006)* fertilization significantly increased the yield of grass-legume mixtures and pure grass crops, but the difference in yields between crops decreased by adding N. So, in variants without fertilization, mixtures of white clover and cocksfoot exceeded yield of pure cocksfoot crops by 1.7 t ha^{-1}, in treatments with 50 kg N ha^{-1} by 0.9 t ha^{-1} and in treatment with 100 kg N ha^{-1} by 0.1 t ha^{-1} . Also, *Nešić et al. (2007)* have confirmed in their research that N fertilization resulted in a significant increase in yield of grass-legume mixtures, especially high nitrogen rates of 210 kg N ha^{-1}.

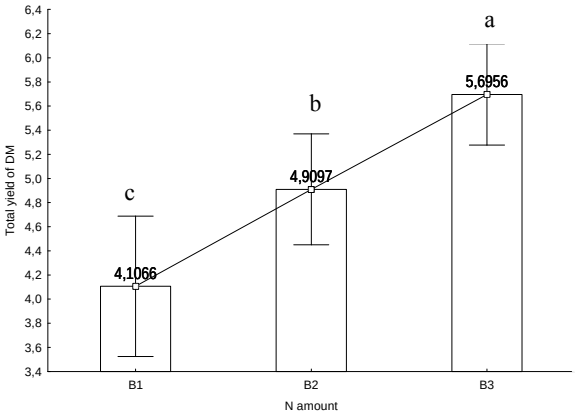

Figure 3. Effect of nitrogen on total dry matter yield t ha^{-1}

The mixture had statistically highly significant ($p \leq 0.01$) impact on the content of CP, NFE NDF, ADF, and ME and statistically significant ($p \leq 0.05$) in NEL in dry weight of fodder (Table 4).

Crude protein amount was the highest (164.9 g kg^{-1}) in pure alfalfa crop (A1) and the lowest (121.2 g kg^{-1}) in the meadow fescue crop (A3). The crops of cocksfoot (A2), English ryegrass (A4), alfalfa +cocksfoot (A5) and alfalfa +cocksfoot+ meadow fescue (A7) have differed significantly in content of crude protein in forage (141.7, 135.2, 126.2 and 135.4 g kg^{-1}, respectively). The presence of alfalfa in mixtures with grasses increased the CP content of the diets as opposed to pure crops, grass, particularly in relation to meadow fescue. According to *Mika et al. (2004)*, legumes in a mixture, in addition to the increase the content of CP should also increase the content of NEL, which was not the case in the present study. In our research, content of net energy for lactation was significantly statistically higher ($p \leq 0.05$) in pure cultures (6.10 MJ kg^{-1} DM in A4) compared to grass-legume mixture. Crops of meadow fescue and English ryegrass had significantly higher content of NFE as compared to the mixtures and in relation to pure alfalfa crop. NDF was the highest in the crop of meadow fescue, and the lowest in alfalfa pure crop. NDF content in mixtures ranged from 521.2 (A6) to 541.2 (A5). *Albayrak et al. (2011)* also concluded in their research that the NDF content was significantly higher in pure grass crops, compared to pure alfalfa crops and alfalfa mixtures, and that the alfalfa in monoculture had the lowest NDF content. Mixtures had significantly ($p \leq 0.01$) higher content of ADF compared to the pure culture, while the content of metabolizable energy was significantly higher in pure cultures from 9.70 (A2 and A3) to 10.11 MJ kg^{-1} DM (A4) than in mixtures where it ranged from 9.46 (A6) to 9.60 MJ kg^{-1} DM (A5).

Table 4. The content of crude protein, crude fiber, fat, ash, NFE, NDF, ADF, ME and NEL in pure crops and their mixtures depending on N fertilization

Treatments	CP	CF	CFT	Ash	NFE	NDF	ADF	ME	NEL
	g kg-$^{-1}$							MJ kg^{-1} DM	
Crop									
A1	164.9^{a}	298.6	26.0	81.2	341.9^{d}	403.2^{g}	327.5^{d}	9.93^{b}	6.00^{b}
A2	141.7^{bc}	303.4	33.2	86.1	365.2^{bc}	467.2^{ef}	343.1^{c}	9.70^{c}	5.87^{c}
A3	121.2^{c}	292.1	26.7	87.5	401.0^{a}	560.2^{a}	343.2^{c}	9.70^{c}	5.87^{c}
A4	135.2^{bc}	281.7	30.4	90.8	396.0^{a}	506.0^{f}	314.8^{e}	10.11^{a}	6.10^{a}
A5	126.2^{bc}	300.9	32.2	81.7	383.6^{ab}	541.2^{b}	350.2^{bc}	9.60^{cd}	5.8^{cd}
A6	142.5^{b}	300.3	27.9	81.2	368.8^{bc}	521.2^{d}	359.8^{a}	9.46^{e}	5.74^{e}
A7	135.4^{bc}	299.8	29.1	81.8	369.7^{bc}	529.4^{cd}	357.2^{ab}	9.50^{de}	5.76^{d}
A8	145.5^{ab}	302.5	29.5	78.1	358.8^{cd}	536.1^{bc}	350.6^{bc}	9.59^{cd}	5.81^{cd}
Level of significance	**	ns	ns	ns	**	**	**	**	*
N fertilization									
B1	137.4	294.1	28.0	85.3	376.1^{b}	503.4^{b}	343.9	9.69	5.86
B2	134.6	292.8	29.7	84.6	382.2^{a}	517.9^{a}	342.3	9.71	5.88
B3	145.2	305.3	30.3	80.8	361.1^{c}	517.8^{a}	343.7	9.69	5.87
Level of significance	ns	ns	ns	ns	**	**	ns	ns	ns

A1- alfalfa; A2 – cocksfoot; A3- meadow fescue; A4-and English ryegrass; A5- 50% alfalfa + 50% cocksfoot; A6- 50% alfalfa, 25% cocksfoot and 25% meadow fescue; A7- 25% alfalfa, 50% cocksfoot and 25% meadow fescue; A8- 40% alfalfa, 20% cocksfoot e, 20% meadow fescue and 20% English ryegrass; B1-0 kgN ha^{-1}; B2-50 kgN ha^{-1}; B3-100 kgN ha^{-1}; **- $p \leq 0.01$; *- $p \leq 0.05$; ns- non significant

For optimum milk production certain content of ME in plants is required. According to *Older et al. (1987)* for the production of 10 kg cow^{-1} day^{-1} of milk, it is necessary that plants contain 6.4 MJ kg^{-1} DM, while for production of double amount of milk 10.2 MJ kg^{-1} DM is needed. According to *Lindgren and Lindberg (1988)* that content is even higher, 11.5 to 12.5 ME kg-1 DM. In the studied crops the required content of ME for intensive dairy production was not reached, which is a sign that certain agricultural practices and measures should be taken to increase energy content of plants through the implementation of the optimal regime of exploitation or cutting (*Lättemäe and Tamm, 1997).*

Nitrogen fertilization had a significant impact on the contents of NFE and NDF while other parameters did not show significant variations between treatments with different amounts of nitrogen. NFE content was the highest in the treatment with 50 kg N ha^{-1} of 382.2 g kg^{-1} DM and NDF content of 517.9 g kg^{-1} DM. Similar to present research, in studies by other scientists NDF content in plants increased significantly with the addition of N fertilizer, which is explained as the

consequence of the reduction in the share of legumes, and increase of the share of grasses with N fertilization (*Kopp et al., 2003 Salis and Vargiu, 2008*).

The amount of nitrogen had no significant effect on the crude protein content in dry forage mass, although the CP content was the highest in the treatment with the highest amount of added nitrogen of 145.2 g kg-1 DM.

Conclusion

The studied crops showed significant differences in achieved yields. Alfalfa in monoculture had the highest yield of dry matter, while the pure grass crops had the lowest yields – the lowest yield was established for English ryegrass of 2.44 t ha^{-1}. Of studied alfalfa mixtures the highest yields were realized by mixtures of alfalfa and cocksfoot (A5) of 5.87 and a mixture of alfalfa, cocksfoot and meadow fescue (A6) of 5.65 t ha^{-1}. The addition of N fertilizer influenced a significant proportional increase of the dry weight of plants. The values of CP, NFE, the NDF, ADF, ME and NEL showed significant variability in relation to the type of crop. CP content was the highest in pure alfalfa crop, and the lowest in meadow fescue crop, whereas in alfalfa mixtures, CP content was at a satisfactory level,126.2 - 141 .7 g kg^{-1}. NDF, ME and NEL contents were significantly higher in pure crops, especially grass crops, compared to grass-legume mixtures, while ADF content was higher in the mixtures. N fertilization had no particular impact on the quality of forage of pure cultures and their mixtures, the only significance of impacts were noted in the content of NFE and NDF. Alfalfa as most grown forage crop, with its yields continues to justify the leading place, though in addition to alfalfa, especially in the lowland regions, could recommend a mixture of alfalfa and cocksfoot, as well as alfalfa, cocksfoot and meadow fescue, due to their advantages and quality and yield of forage that can meet the needs of producers with proper fertilization.

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Efekat N djubrenja na prinos i kvalitet trava i leguminoza u monokulturi i u smeši

Z. Tomić, Z. Bijelić, V. Mandić, A. Simić, V. Caro-Petrović, M. Žujović

Rezime

Ogled je izveden na oglednom polju Instituta za stočarstvo u periodu 210-2012. godine. Istraživanjima su obuhvaćeni čisti usevi lucerke (A1), ježevice (A2), livadskog vijuka (A3) i engleskog ljulja (A4) kao i njihove smeše u različitim konbinacijama (50% lucerke i ježevice - A5, 50% lucerke, 25% ježevice i 25% livadskog vijuka - A6, 25% lucerke, 50% ježevice i 25% livadskog vijuka - A7 i 40% lucerke, 20% ježevice, 20% livadskog vijuka i 20% engleskog ljulja - A8). Đubrenje N radjeno je rano u proleće, sa kretanjem vegetacije u tri tretmana: 0 (B1), 50 (B2) i 100 kg ha^{-1} (B3).

Cilj ovih istraživanja je da se ispita uticaj različitih količina N iz mineralnih đubriva na proizvodne, kvalitativne i energetske karakteristike krmnih useva kao monokulture i kao smeše. Usev kao ispitivani faktor imao je statistički značajnog uticaja na prinos suve mase i sadržaj CP, NFE NDF, ADF, ME i NEL, a N đubrenje na prinos i sadržaj NFE-a i NDF-a.

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HEALTH STATUS CONTROL OF WILD BOARS IN THE HUNTING AREA OF VOJVODINA REGION

J. Prodanov-Radulović, R. Došen, I. Pušić, T. Petrović, I. Stojanov

Scientific Veterinary Institute «Novi Sad», Rumencki put 20, Novi Sad,
Republic of Serbia
Corresponding author: jasna.prodanov@gmail.com
Original scientific paper

Abstract: In Vojvodina region a certain number of wild boars is controlled and reared in an enclosed hunting ground while the number of free-ranging population is mainly unknown. One of the characteristics of outdoor swine production is raising free-roaming domestic pigs, where they share forest habitat with wild boars. The aim of the research was health status control of wild boars population in the hunting area of Vojvodina region. The applied research methods included: epizootiological analysis, pathomorphological examination of dead wild boars and laboratory testing in order to detect the infection with the classical swine fever virus (CSFV) and infection with *Brucella* sp. In order to diagnose diseases, the pathomorphological examination of 35 carcasses of shot or diseased animals was performed. Patomorphological examination revealed that health problems in wild boars were mainly associated with parasitic infections and bacterial infections of the lungs. In examined organs and tissue samples deriving from dead and shot wild boars the presence of CSFV was not detected. By serological testing, the antibodies against *Brucella sp*. in 7 samples were detected. The achieved results suggest that the presented way of control should be intensified in future in order to acquire complete insight in health status of wild boars. Our study underlines the importance of improving surveillance strategies for pathogens shared between wildlife and domestic animals and the need to increase disease awareness of farmers and veterinary practitioners.

Keywords: health status control, wild boars

Introduction

Wild boar (*Sus scrofa scrofa*) numbers have dramatically increased in European countries over the past 60 years and the species shows a more widespread distrbution (*Artois et al., 2002*). The parallel increase of outdoor piggeries has led to a higher risk of contacts, and thus of disease transmission

between wild boars and domestic pigs (*Prodanov et al., 2009; Wu et al., 2011*). The overabundance of wildlife, recognized as a relevant risk factor for disease transmission between wildlife and domestic animals, compromises the health surveillance programs carried out both populations (*Gregoire et al., 2012*). Knowledge of diseases circulating in wildlife populations can be important not only for conservation and livestock production but also for public health (*Ruiz-Fons et al., 2008*). The aim of the research was health status control of wild boars population in the hunting area of Vojvodina region.

Material and Methods

The material for this research included wild boars in hunting areas of Vojvodina region. The applied research methods included: anamnestical and epizootiological data analysis, pathomorphological examination of shot, diseased or dead wild boars (in total 35 carcasses). Laboratory testing of tissue and blood samples in order to detect the infection with the classical swine fever virus (CSFV) (ELISA test and RT-PCR) and infection with *Brucella sp.* (Rose Bengal Test, Complement Fixation Test and cELISA test) were performed. Standard bacteriological laboratory testing included detection the precence of aerobic and anaerobic bacteria in the tissue samples. Beside this, each animal was throughly analysed for the presence of helminths (parasitological testing). Lung, digestive tract, liver, gall-bladder and renal pelvis were examined with routine techniques for the detection of helminth parasites. A coprological analysis was carried out with faecal material extracted from the rectum of each animal after necropsy by zinc sulfate flotation and sedimentation.

Results and Discussion

In cooperation with the hunting societies and local veterinary service gathering of sera samples of hunted wild boars was organised. Serological examination in year 2009 on the presence of specific antibodies against CSFV (ELISA test) comprised only 259 blood samples obtained from wild boars in the hunting area of Vojvodina region and it revealed negative result. In year 2010 the serological control of CSFV antibodies in wild boars population included significantly larger number of animals i.e in total 471 tissue samples and 455 blood samples were examined. From examined blood samples, in 36 positive result i.e the presence of specific antibodies against CSFV was detected. However, applying RT-PCR analysis the presence of viral genome was not established in tissue samples deriving from shot wild boars. Applying epizootiological evaluation, it

was discovered that only 25 examined sera samples were from wild boars vaccinated against CSF in the past. The epizootiological and serological active control of the CSFV presence in wild boars population in Vojvodina region was even more intensified during years 2011-2012. In total 2038 samples were examined: 996 sera samples and 1042 tissue samples (spleen, lympho node, kidney). Once again, in 33 sera samples the presence of CSFV specific antibodies was detected. By epizootiological evaluation, it was discovered that during 2009-2010 year in some of the hunting grounds vaccination against CSFV was applied, with modified live (China strain) vaccine. This could explain the presence of specific antibodies against CSF virus in examined sera samples. However, the results of the epizootiological questionnaire indicated that CSFV may be present in hunting grounds in the region of Danube river, implying that the wild boars population represents also a source of infection with CSFV (*Prodanov et al., 2009; Maljković et al., 2010*).

CSFV circulates among the wild boar populations of Central and Eastern Europe but most of Western Europe is considered to be CSF-free (*Artois et al., 2002*). Epidemiological links between CSFV infections in wild boars and domestic pigs have been repeatedly reported, mainly in Germany. In countries members of the European Union, vaccination against CSF is not permitted and the stamping out policy is conducted in case of an outbreak (*Ruiz-Fons et al., 2008*). Our country is among small number of countries that still perform vaccination in pigs with conventional (China strain) vaccines.

In 7 samples deriving from shot wild boars from one hunting area the presence of antibodies against *Brucella sp.* was detected, demonstrating the circulation of this bacteria amongst wild boar population. Seropositive wild boars in enclosed hunting ground represent a significant health problem both for hunters and for consumers of meat and for the population of domestic pigs.

Swine brucellosis (*Brucella suis* biovar 2), is one of the most important endemic diseases in the wild boar populations in Central Europe (*Bergagna et al., 2009*). In wild boar, *B. suis* is transmitted mainly by the veneral route (mating) and may be inapparent among living animals. Furthermore, metritis can easily be overlooked at slaughter, and infections are not always associated to pathological changes. Intrusions of wild boar and mating with domestic sows have been documented in France, Germany, Sardinia and Switzerland and numerous populations of hybrids are established (*Wu et al., 2012*). Avoiding close contact between wild boars and domestic animals is therefore of logical importance in disease control and eradication programmes (*Ruiz-Fons et al., 2008; Wu et al., 2011*).

In Vojvodina region a certain number of wild boars is controlled and reared in an enclosed hunting ground while the number of free-ranging population is mainly

unknown. One of the characteristics of outdoor swine production is raising free-roaming domestic pigs, what enables their interaction with wild boar population because they share forest habitat with wild boars (*Prodanov et al., 2009; Prodanov-Radulović et al., 2010*). Having in mind this fact, the special attention should be given to active surveillance of wild boars population in the areas where close contact with domestic swine is possible. The recent isolation of *B. suis* biovar 3 from pigs, wild boar and horses in Croatia shows the emergence of zoonotic biovars in Europe. Translocation of wild boar for breeding or hunting purposes increases the risk of spreading of zoonotic brucellosis throughout Europe. It remains important to biotype *Brucella* strains isolated from wildlife in surveillance programs throughout Europe (*Gregoire et al., 2012*).

As part of health control programm of clinically diseased wild boars shot by hunters, pathoanatomical examination of trunci and internal organs deriving from 35 wild boars was performed. The pathomorphological examination of clinically suspected i.e. diseased animals (emaciated wild boars, slow gait, lagging behind the pack, dispnoea, nasal discharge, shrunken eyes and tough dry hair), shot by hunters revealed changes dominantly in the respiratory tract: purulent nasal discharge, effervescent content in bronchi and bronchioles, mixed with a large number of lung worms, which were like mucoid plugs filling the respiratory pathways. All lobes of the lungs were diffusely swollen, edematous and reddened with marginal emphysema and consolidation. A large amount of clear, foamy fluid and numerous slender, white nematodes 4-7 cm long were visible in the trachea and bronchial trees. The presence of lung worms (*Metastrongylus spp*.) in the trachea, bronchi and in posteroventral parts of the diaphragmatic lobes were detected. Applying parasitological control of the faecal material extracted from the rectum the presence of the several parasites was discovered: *Trichuris suis, Oesophagostomum sp.* i *Hyostrongylus sp*. By standard bacteriological testing on tissue samples (lungs, lympho nodes, liver, spleen, kydnes) the following bacteria was detected: *Actinobacillus pleuropneumoniae, Streptoccocus alfa haemolyticus, Streptococcus beta haemolyticus, Pasteurella multocida.*

Lungworms are often encountered as highly prevalent helminthes in wild boars (*Prodanov-Radulović et al., 2011*; *Senlik et al., 2011*). In Europe, these parasites have a high prevalence, affecting more than 80% of pigs created in extensive system and considered one of the main causes of respiratory changes of these animals (*da Silva et al., 2013*). Similarly, a high prevalence rate of metastrongylids was also recorded in the present study. This result might be explained by the wide geographical distribution of different earthworm species, which form part of the diet of wild boars and act as intermediate hosts for these parasites (*Senlik et al., 2011*). Lung parasites of the genus *Metastrongylus* are considered one of the most important selective factors acting on wild boar

population, increasing the mortality of weaker young and adult animals because they may cause dyspnea, bronchopneumonia, and permanent weight loss in addition to inflicting tissue damages which allow opportunistic infections of viruses and bacteria (*da Silva et al., 2013*). Despite the limited number of wild boars examined, our study suggests these species are common and enzootic in wild boars in Vojvodina region.

Conclusion

Patomorphological examination carcasses of shot, found dead or diseased animals shoth by hunters originating from different hunting areas of Vojvodina revealed that health problems in wild boars were mainly associated with parasitic infections and bacterial infections of the lungs. In conclusion, the achieved results suggest that the presented way of control should be intensified in future in order to acquire complete insight in health status of wild boars. Our study underlines the importance of improving surveillance strategies for pathogens shared between wildlife and domestic animals and the need to increase disease awareness of hunters, farmers and veterinary practitioners.

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Kontrola zdravstvenog statusa divljih svinja u lovištima regiona Vojvodine

J. Prodanov-Radulović, R. Došen, I. Pušić, T. Petrović, I. Stojanov

Rezime

U regionu Vojvodine određeni broj divljih svinja se kontrolisano odgaja u ograđenim lovištima dok je tačan broj jedinki u slobodnim staništima uglavnom nepoznat. Sa druge strane, jedna od karakteristika ekstenzivnog uzgoja domaćih svinja u pojedinim regijama jeste pašnjački način gajenja, gde one dele stanište i

dolaze u neposredan kontakt sa divljim svinjama. Cilj rada je bio ispitivanje zdravstvenog statusa populacije divljih svinja u lovištima na području Vojvodine. Primenjene metode ispitivanja su obuhvatale: epizootiološka analiza i patomorfološki pregled odstreljenih i uginulih divljih svinja kao i laboratorijska ispitivanja u cilju utvrđivanja infekcije sa virusom klasične kuge svinja i *Brucela sp*. U cilju zdravstvene kontrole i dijagnostike oboljenja, izvršen je patomorfološki pregled 35 trupova uginulih i na oboljenje sumnjivih odstreljenih divljih svinja. Patomorfološkim pregledom ustanovljeno je da su zdravstveni problemi kod divljih svinja vezani uglavnom za parazitske infestacije i pneumonije bakterijske etiologije. U ispitanim uzorcima organa i tkiva poreklom od uginulih i odstreljenjih divljih svinja nije utvrđeno prisustvo virusa KKS. Sreološkim ispitivanjem, u 7 uzoraka krvnih seruma utvrđeno je prisustvo antitela protiv *Brucella sp*. Postignuti rezultati ispitivanja ukazuju da predstavljeni način kontrole treba u budućnosti nastaviti da bi se postigao što kompletniji uvid u zdravstveno stanje divljih svinja. Rezultati ukazuju na značaj zdravstvenog nadzora divljih i domaćih životinja i potrebu za strateškom saradnjom lovaca, farmera i veterinarske zdravstvene službe.

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PRODUCTION RESULTS OF RING-NECKED PHEASANT PARENTS FLOCKS DEPENDING OF NUTRITION ON FARM AND YEAR

Z. Popović[1], B. Bojović[2], N. Đorđević[1], D. Beuković[3], M. Beuković[3], M. Đorđević[4]

[1]Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Zemun, Republic of Serbia
[2]Hunting association Čačak, Republic of Serbia
[3]Faculty of Agriculture, University of Novi Sad, Trg Dositeja Obradovića 8, 21000 Novi Sad, Republic of Serbia
[4]Faculty of Veterinary Medicine, University of Belgrade, Bulevar oslobođenja 18, Republic of Serbia
Corresponding author: nesadj@agrif.bg.ac.rs
Original scientific paper

Abstract: The parent flocks of ring-necked pheasant in experiment on two different farms were tested production results for period 2008/2010 year. The sex ratio at the beginning of the reproductive season on both farm was 1:7. Out reproductive period parent flocks were fed concentrate diets with 18% CP. During reproductive period on 1st farm used a mixture of concentrate with 22% CP and at the 2nd farm with 20% CP. Using mixture containing 22% crude protein on 1st farm results was determined the average number of laying eggs in the season 37.17eggs and 0.44 eggs per hen by day. Percent hatched of brooding eggs in season was 64.83%, hatched by pheasant hen in season was 21.44. On 2nd where used 22% crude protein in diet average number of laying eggs in the season was 55.51 eggs and 0.54 eggs per hen by day. Percent hatched of brooding eggs in season was 60.53%, hatched by pheasant hen in season was 32.16.There was determined a significance for „Farm" variable ($p<0.05$) for average number of laying eggs in season. Variable „Year" was not significant to production results. Better load egg per pheasants on 2nd farm is attributed to the improved genetic potential of breeding flocks on that farm. In order to increase the number of hatched of brooding eggs in season recommended the introduction ovary-scoping of eggs.

Key words: pheasant, parent flock, production results.

Introduction

There are several important factors that have a negative impact to the number of pheasant in hunting grounds: reduced areal, deteriorating natural resources, food, machinery, and agricultural chemicals use, predators, diseases, transport, high percentage shooting and others *(Popović et al., 2008, 2011; Popović and Đorđević, 2010; Djordjevic et al., 2012a)*. In such circumstances pheasant population cannot be naturally renewed, and human intervention is necessary. Experimental trials with additional ring-necked pheasant feeding in the nature, during spring breeding period did not contribute to improving the reproductive results *(Hoodles et al., 2001)*. Summer feeding of young ring-necked pheasant that are populated in the hunting ground, there positively influenced to body condition and survive overwinter, but it's not enough measures to regulate the number of this game *(Sage et al., 2002)*. The only effective way to maintain the number of ring-necked pheasant is controlled reproduction in rearing and settling produced offspring in the hunting grounds with the appropriate age and after appropriate adjustment *(Đorđević et al., 2011a,b)*.

Serbia has over thirty registered pheasant farm with a capacity that exceeds current needs of all hunting *(Popović et al., 2011)*. Operating techniques and equipment is often outdated so do not give satisfactory results. Due to the influence of several factors on the production results achieved, there are significant differences between the pheasant farm. This study followed reproducible results flock of pheasant farm on two different, with different production conditions.

Material and Methods

The analysis of ring-necked pheasant production was performed in pheasant farms „Šumarice" (the pheasant farm 1) and „Vranjica" (pheasant farm 2) over three years (2008-2010.). The sex ratio of pheasant farms was the same, 1:7. In the period beyond reproduction, the parent flocks were fed concentrate diets with 18% CP, and from playing on the farm 1 used a mixture of concentrate with 22% CP and the farm 2 with 20% CP. The chemical composition of the mixture is shown in Table 1. Feeding was ad libitum and the average feed daily consumption on the pheasant farm 1 was 80 g , vs pheasant farm 2 was 70-80 g.

Table 1. Chemical composition of concentrate mixtures for ring-necked pheasants in the reproductive season, %

Parameters	Pheasant farm 1	Pheasant farm 2
Dry matter (DM)	87	87
Crude protein (CP)	22	20
Crude fiber (CF)	8	5
Ash	10	12
Ca	2.2	2.4
P	0.8	0.7

Pheasant farm 1 - pheasant farms "Šumarice"; Pheasant farm 2 - pheasant farms "Vranjica"

During the summer in pheasant farm 1, eggs were collected every hour (from seven in the morning) and kept in the sand. Set the eggs in the incubator was done in on days 5-10. In the incubator station, are four incubators Italian company „Victoria", a capacity of 2×4,620 and 2×7,050 eggs. In the pheasant farm 2 collecting eggs was conducted in the same manner as in the previous case and storage in cardboard boxes. Eggs are invested every 7 days in incubators domestic production of brand „Morava", capacity of 4,000 to 2,000 eggs.

The production parameters of parents flock were observed: average number of laying eggs by season and average number of egg per pheasant hen daily. Results hatching chickens were observed through: percent brooding eggs of layed eggs, percent hatched of brooding eggs in season, hatched by pheasant hen in season . Statistical analysis was performed software *Statsoft (2012)*.

Results and Discussion

During observed period it was found significantly higher number of laying eggs in season for pheasant farm 2 ($p<0.05$), and average number of egg per pheasant hen daily (Table 2). It should be noted that in the reproductive season in the breeding pheasant farm 2, used mixtures with lower protein content (20%). Variations in the percent brooding eggs of laid eggs at the farms in the study period was not significant. The determined values for average number of laying eggs in season in the three-year period (45.59-47.08) are slightly higher than the state *Urošević (2005)*. The egg production in local conditions (season 90 days, the period of April-June), it's 41-45 eggs. Pheasants in nature, carry 12-18 eggs weight 28-29 g per egg, while in the aviary can lay up to 60 eggs for about 3 months *(Popović and Đorđević, 2009; Đorđević et al., 2011b)*. *Bojović (2012)* states that the capacity of pheasants in the experimental conditions was up to 100 eggs in a season, and in conditions of extended even 140 days. To the characteristics of hatching eggs affects a lot of factors: genetic structure of breeding stock, rearing, feeding, age, and duration of the season pheasants capacity *(Essen et al., 2010)*.

This can explain the significantly higher difference in this parameter between the observed production pheasant farm (55.51:37.17).

Nutrition is one of the important factors that affect the number of eggs laid *(Carey et al., 1980)*, their weight *(Usturoi, 2008)* and fertility *(Nowaczewski and Kontecka, 2005)*. As reported by *Đorđević et al. (2013)*, food affects the number and size of eggs laid directly over the necessary nutrients to provide a meal, or indirectly through the body's reserves, which are provided in the past. However, nutrients meals such as protein and energy sources have a much greater effect on the formation of eggs than to hen fertility, in which the dominant role is played by vitamins and minerals *(Đorđević et al., 2009)*.

Percent brooding eggs of laid eggs was also higher pheasant farm 2. Ovary-scoping of eggs before being placed in incubators is an important measure for elimination of unfertilized eggs, and its increasing the percentage of hatched eggs. However, in the observed pheasant farms ovary-scoping have not done, and grading eggs based on their weight and shape, which is a major drawback. This method of classification of eggs is a direct consequence of the percentage of hatched, which was less in pheasant farm 2 ($p>0.05$) and very small for both pheasant farm (64.83 and 60.53). The percentage of hatched eggs is considered satisfactory if 85% of the fertilized eggs hatch, although this percentage may under favourable conditions be higher *(Bojović, 2012)*.

Table 2. Production results of pheasant parent flock depending on the pheasant farm and year

Pheasant farm	*Year*	*Average number of laying eggs in season*	*Average number of egg per pheasant hen daily*	*Percent brooding eggs of layed eggs*	*Percent hatched of brooding eggs in season*	*Hatched by pheasant hen in season*
Pheasant farm 1 (22% CP)	2008.	39.55	0.44	89.60	68.51	24.28
	2009.	34.67	0.46	84.71	67.08	19.70
	2010.	37.28	0.43	83.22	58.89	20.35
Pheasant farm 2 (20% CP)	2008.	53.16	0.51	93.19	72.08	35.71
	2009.	59.48	0.59	98.27	53.73	31.40
	2010.	53.90	0.53	97.68	55.77	29.36
Average for 2008.		46.36	0.48	91.40	70.30	30.00
Average for 2009.		47.08	0.52	91.49	60.40	25.55
Average for 2010.		45.59	0.48	90.45	57.33	24.86
Average for farm 1		37.17	0.44	85.84	64.83	21.44
Average for farm 2		55.51	0.54	96.38	60.53	32.16
Significance for pheasant farm		0.014288^{*}	-	-	0.225205^{ns}	-
Significance for year		0.259221^{ns}	-	-	0.223030^{ns}	-
Significance for interaction of factors		0.250971^{ns}	-	-	0.121705^{ns}	-

Pheasant farm 1 had a slightly lower percentage of hatched pheasants the pheasants in the season. These results are also due to the influence of many different factors to the percentage hatching. So, for example, the success of incubation depends on the biological quality of the eggs, incubators and modes of engagement of people. Biological quality is related to the fertility of eggs, the method and length of storage and proper selection of hatching eggs. On fertility affects the structure flock, rearing, and sex ratio. The influence of diet on the biological quality of the eggs is correlated with the content of vitamins and minerals *(Greeley, 1962; Hinkson et al., 1979; Suchy et al., 2008)*. Fertility of eggs in nature and can be 96%, but due to large losses (up to 85%) real growth per pheasant hen is very small *(Bojović, 2012)*. To the fertility may adversely affect cold and rainy periods, the presence of intruders and disturbance and of parents flock *(Popović and Stanković, 2009)*. Fertility is lower at the beginning and end of the season hatching.

Conclusion

Improvement of breeding technology at pheasant's farms and settlement of their offspring in hunting grounds enabled the efficient maintenance of the quantity of this kind of game in terms of significantly altered living conditions. The nutrition of parents flock has an important role to achieve maximum production output and total weaned pheasants per hen. Beside nutrition status, lot of other factors influence on performance, which in this experiment led to a large difference between the pheasant farm in the studied parameters. In the future, we should work on improving a number of factors that are crucial for the results which relate to the technical equipment of the pheasant farm, and on the selection measures in their respective flocks.

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Proizvodni rezultati matičnog jata fazana u zavisnosti od ishrane na farmi i godine

Z. Popović, N. Đorđević, D. Beuković, M. Beuković, M. Đorđević

Rezime

U eksperimentu su ispitivani proizvodni rezultati matičnog jata fazana na dve farme za period 2008-2010. Odnos polova na početku reproduktivne sezoni na farmama je bio 1:7. U periodu van reprodukcije matična jata su hranjena smešama koncentrata sa 18% SP, a u periodu reprodukcije na farmi 1 korišćena je smeša koncentrata sa 22% SP a na farmi 2 sa 20% SP.

Na farmi 1 i pri ishrani smešom koncentrata sa 22% sirovih proteina utvrđena je prosečana nosivost od 37,17 jaja u sezoni, 0,44 jaja po fazanki dnevno, 64,83 procenata izleženih od nasađenih jaja u sezoni i 21,44 izleženih po fazanki u sezoni. Na farmi 2 i pri ishrani smešom koncentrata sa 20% sirovih proteina utvrđena je prosečana nosivost od 55,51 jaja u sezoni, 0,54 jaja po fazanki dnevno, 60,53 procenata izleženih od nasađenih jaja u sezoni i 32,16 izleženih po fazanki u sezoni. Utvrđena je signifikantnost faktora farme ($p<0,05$) za nosivost u sezoni. Faktor godine nije signifikantno uticao na proizvodne rezultate. Bolja nosivost fazanki na farmi 2 pripisuje se boljem genetičkom potencijalu priplodnog jata. U cilju povećanja broja izleženih od broja nasađenih preporučuje se uvođenje ovoskopiranja jaja.

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DETERMINATION OF TOXIC ELEMENTS IN WILD BIRDS FROM THE AREA OF VOJVODINA

Ž. Mihaljev, M. Živkov-Baloš, M. Kapetanov, S. Jakšić

Scientific Veterinary Institute „Novi Sad“, Rumenački put 20, 21000 Novi Sad, Republic of Serbia
Corresponding author: zeljko@niv.ns.ac.rs
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Abstract: Content of lead, cadmium, arsenic and mercury was examined in 24 samples obtained from the following wild birds: Swan *(Cygnus)*, March hen (*Gallinula choloropus)*, Little egret ***(Egretta garzetta)***, Buzzard eagle (**Buteo buteo)**, White-tailed eagle (*Haliaeetus albicilla)*, Seagull (*Larus ridibundus*), White stork (*Ciconia ciconia*), Wild mallard (*Anas platyrhynchos*), Grey heron (*Ardea cinerea*). The samples of muscle tissue, liver, kidneys and heart tissue were analyzed. The samples were prepared by wet digestion using Ethos, Labstation Microwave, Milestone. Lead, cadmium, arsenic and mercury were analyzed by the method of coupled plasma on the Agilent ICP-MS 7700. The highest lead contents were registered in the liver and kidneys of the examined birds, with average values of 0.372 ± 0.290 mg/kg and 0.384 ± 0.168 mg/kg, respectively. The highest cadmium concentrations were recorded in the same organs, being averagely 0.083 ± 0.077 mg/kg in the liver and 0.248 ± 0.052 mg/kg in the kidneys. The content of arsenic was very low in all investigated samples (< 0.10 mg/kg). In some samples, significantly increased mercury content was established. The levels of mercury in the meat of grey heron, liver of white-tailed eagle and kidney of white-tailed eagle were 0.717 mg/kg, 1.128 mg/kg and as much as 3.656 mg/kg, respectively.

Key words: lead, cadmium, arsenic, mercury, wild birds

Introduction

In order to fully understand the exposure of animals to the pollutants originating from the environment and to assess their harmful effects and estimate the risk, it is necessary to carry out a systematic study and collect data on the degree and type of pollution, as well as distribution of hazardous chemicals in the environment. Nowadays, a number of studies have been focused on determination of chemical contaminants in animal tissues and organs *(Milošević and Vitorović, 1992)*. The obtained results enable the assessment of human exposure to negative effects of these pollutants.

Many wild animals are exposed to different toxic substances by consuming contaminated plants and animals, water, soil and air *(Saičić et al., 1995)*. Since animals can move freely and find their own food, the game (including wild birds) is a link in the chain that accumulates pollutants from the environment. Therefore, wildlife species in certain geographic areas may represent a good indicator of environmental pollution, especially certain chemical elements, as they eat unprocessed plants in a particular habitat *(Mihaljev et al., 1990, 1991)*. It should be noted that the accumulation of chemical elements is affected by endogenous factors (age, sex, health status of animals) and exogenous factors (geography, hydrological conditions, soil, climate, plant life). The importance of this issue is proved by the fact that a completely new discipline is being developed - wildlife toxicology - which includes examining the effects of toxins on wildlife *(Živanov, 2001)*. Recently, comprehensive analysis of the extent and structure of mortality in protected endangered wild animals was performed. The results of pathoanatomical and biological examination strongly suggested that man is directly or indirectly responsible for the mortality *(Kapetanov et al., 2012)*.

The consequence of technological development is general pollution of the environment resulting from a range of contaminants. Ingestion of small amounts of a toxic substance over a longer period results in its accumulation in various tissues, causing chronic poisoning and inducing different diseases and even death *(Pavkov et al., 1993; Mašić et al., 2001)*. Thus, the main objective of this study was to determine the number and type of chemical elements accumulated in the samples of wild birds, and, based on these results, determine the sites with increased content of chemical contaminants. This would enable identification of increasing contaminant levels and determination of appropriate corrective aimed at reducing environmental contamination by chemical agents with the purpose of improving the environment protection and preserving protected and highly protected wild animals.

Material and Methods

A total of 24 samples were tested on the content of trace elements in wild birds: Swan *(Cygnus)*, March hen (*Gallinula choloropus)*, Little egret *(Egretta garzetta)*, Buzzard eagle (**Buteo buteo)**, White-tailed eagle (*Haliaeetus albicilla)*, Seagull (*Larus ridibundus*), White stork (*Ciconia ciconia*), Wild mallard (*Anas platyrhynchos*), Grey heron (*Ardea cinerea*) *(Wüst , 1970)*. Samples of muscle tissue, liver, kidneys and heart collected from wild birds were individually analyzed. The samples were prepared by wet digestion using Ethos, Labstation Microwave, Milestone. Manganese, iron, copper and zinc were determined using atomic absorption spectrophotometry on the Varian Spectraa-10. Nickel, cobalt and

selenium were analyzed by a technique of coupled plasma on the Agilent ICP-MS 7700. Considering the lack of referent values for the maximum permissible levels of metals and non-metals in tissues of wild species, the obtained results were evaluated and compared applying the maximum permissible levels of particular contaminant in animal feed *(Pravilnik, 2011)*.

Results and Discussion

The results of our research are presented in Tables 1 and 2.

Table 1. Contents of toxic elements in the examined samples from wild birds

No	Type of sample	Analyzed elements			
		Pb mg/kg	**Cd** mg/kg	**As** mg/kg	**Hg** mg/kg
1.	Swan – meat	0.096	0.008	0.022	0.028
2.	March hen – meat	0.083	0.006	0.063	0.014
3.	Little egret - meat	0.078	0.003	0.006	0.717
4.	Swan - meat	0.089	0.002	0.016	< 0.001
5.	Swan - meat	0.066	0.008	0.016	< 0.001
6.	Buzzard eagle male – meat	0.125	0.004	0.003	0.050
7.	Buzzard eagle male - liver	0.888	0.047	0.003	0.119
8.	Buzzard eagle male - kidney	0.629	0.328	0.005	0.113
9.	Buzzard eagle female-meat (meat)	0.092	0.013	0.010	0.148
10.	Buzzard eagle female - liver (liver)	0.262	0.201	0.008	0.418
11.	Buzzard eagle female-kidney	0.402	0.238	0.003	0.089
12.	White-tailed eagle – meat	0.108	0.007	0.003	0.450
13.	White-tailed eagle – liver	0.265	0.024	0.002	1.128
14.	White-tailed eagle – kidney	0.430	0.228	0.010	3.656
15.	White stork – meat	0.100	0.003	0.008	0.179
16.	White-tailed eagle – meat	0.075	0.001	0.022	0.437
17.	White-tailed eagle – liver	0.200	0.023	0.013	0.824
18.	White-tailed eagle – kidney	0.269	0.185	0.014	0.931
19.	White-tailed eagle – heart	0.146	0.003	0.012	0.530
20.	Seagull - muscle	0.075	0.013	0.058	0.136
21.	Wild mallard - muscle	0.073	0.002	0.010	< 0.001
22.	Grey heron – meat	0.077	0.008	0.004	0.426
23.	Grey heron – liver	0.243	0.118	0.008	2.132
24.	Grey heron - kidney	0.192	0.259	0.010	0.634

The contents of lead, cadmium, arsenic and mercury determined in the examined samples are displayed in Table 1. Notably high lead contents were observed in male Buzzard eagle, being 0.125 mg/kg in meat, 0.888 mg/kg in the liver and 0.629 mg/kg in the kidneys. Moreover, increased lead contents were

found in the meat of White-tailed eagle, being 0.108 mg/kg. We used the maximum permissible levels of metals and non-metals in feed *(Pravilnik, 2011)* as the "guideline" permissible values, which imply maximum permitted contents of lead in meat and entrails of 0.10 mg/kg and 0.50 mg/kg, respectively.

Maximum permissible content of cadmium in meat is 0.050 mg/kg, whereas contents in the entrails are 0.50 mg/kg and 1.0 mg/kg in liver and kidneys, respectively *(Pravilnik, 2011)*. Data presented in Table 1 indicate that kidneys are critical organ in a view of cadmium content, as the highest cadmium level was determined in the kidney of Buzzard eagle, reaching as high as 0.328 mg/kg. A group of authors *(Saičić et al., 1995)* reported similar values of kidney cadmium obtained in their research. In all meat samples examined, the content of cadmium was significantly below the maximum permissible values, which corresponds with our former research *(Kljajić et al., 1994)*.

Highest arsenic contents were established in meat of March hen (0.063 mg/kg), Seagull muscle tissue (0.058 mg/kg) as well as in the meat of Swan (0.022 mg/kg) and White-tailed eagle (0.022 mg/kg). Arsenic contents determined in all other meat samples and all liver and kidney samples were very low, below 0.02 mg/kg. Since the maximum permissible contents of arsenic in meat and entrails *(Pravilnik, 2011)* are 0.1 mg/kg and 0.5 mg/kg, respectively, we may conclude that examined samples of wild birds did not manifest any significant arsenic contamination. This is in accordance with the results reported by other authors *(Saičić et al., 1995)*.

If maximum permissible levels of metals and non-metals in feed *(Pravilnik, 2011)* are considered the "guideline" permissible values, the maximum permissible mercury contents are 0.03 mg/kg in meat and 0.10 mg/kg in the entrails. According to data displayed in Table 1, highly increased mercury content was determined in 80% of examined samples. Only in five samples, the content of mercury was within the ranges that could be considered normal. It is important to emphasize that mercury levels measured in some samples exceed the established guideline values by several times. Thus, mercury content in Grey heron meat was 0.717 mg/kg, which is 24 times higher than the maximum permissible value. Mercury contents in the liver of Grey heron (2.132 mg/kg) and in kidneys of White-tailed eagle (3.656 mg/kg) were 21-fold and 37-fold higher than the maximum permissible guideline values, respectively.

Table 2: Total number of analysed samples (n), the mean value of the elements in different sample types (σ) and the interval of measured values (Iv)

No	Type of sample	Analyzed elements			
		Pb mg/kg	**Cd** mg/kg	**As** mg/kg	**Hg** mg/kg
1.	Meat	n=13 σ=0.088 ± 0.016 Iv=0.073 - 0.125	n=13 σ=0.006 ± 0.004 Iv=0.001- 0.013	n=13 σ=0.018 ± 0.017 Iv=0.010 - 0.063	n=13 σ= 0.199 Iv=<0.001-0.717
2.	Liver	n=5 σ=0.372 ± 0.290 Iv=0.200 - 0.888	n=5 σ=0.083 ± 0.077 Iv=0.023 - 0.201	n=5 σ=0.007 ± 0.004 Iv=0.002 - 0.013	n=5 σ=0.924 ± 0.777 Iv=0.119 - 2.132
3.	Kidneys	n=5 σ=0.384 ± 0.168 Iv=0.192 - 0.629	n=5 σ=0.248 ± 0.052 Iv=0.185 - 0.328	n=5 σ=0.008 ± 0.004 Iv=0.010 - 0.014	n=5 σ= 1.085 Iv=0.089 – 3.656
4.	Heart	n=1 0.146	n=1 0.003	n=1 0.012	n=1 0.530

According to the results presented in Table 2, the highest contents of lead, cadmium and mercury were established in kidneys of examined wild species, with average values being: Pb = 0.384 mg/kg, Cd = 0.248 mg/kg and Hg = 1.085 mg/kg. The content of this contaminant was very high also in the liver, being: Pb=0.372 mg/kg, Cd=0.083 mg/kg and Hg=0.924 mg/kg that fully corresponds with the existing data on bioaccumulation of these toxic elements *(Puls, 1990)*. Upon resorption into blood, these heavy metals are very quickly transported to tissue and organ cells (*Kastori, 1997).* They are not biogenic and their effects are exclusively toxic. Tolerance of the body to these metals is strongly dependant on their concentration, mutual relationship and presence of other microelements (Zn, Cu, Se, Fe) that may reduce or prevent their toxic effects and that positively affect the detoxification process *(Šarkanj et al., 2010)*. Arsenic content that was determined in the analyzed samples of meat, liver, kidneys and heart muscle was very low, being averagely below 0.02 mg/kg in all sample types.

Our analysis furthermore confirmed apparent presence of most toxic elements (Pb, Cd, As, Hg) at detectable levels in all types of investigated samples. Other authors *(Mihaljev et al., 1997; Brajković et al., 2010)* also reported presence of increased levels of heavy metals and other microelements as well as other chemical pollutants, e.g. pesticide carbofuran. The aforementioned results strongly indicate that establishing high levels of diverse residues in tissues of wild animals put forward not only the issue of animal health, but also a wider ecological aspects and potential threat for human health considering that wild birds represent an important and valuable bioindicator of environmental pollution.

In that respect, results of analysis of meat, liver and kidney samples from Grey heron are particularly interesting, as they revealed particularly high contents

of some microelements *(Mihaljev et al., 2012),*predominantly of mercury. Elevated levels of lead in meat and liver of examined eagles, as well as extremely high mercury content in all organs of these birds strongly suggest the contamination of the environment with these pollutants. Thus, it would be of great importance to perform the assessment of the area of origin of these wild birds and to determine the potential sources of such contamination. Increased concentrations of lead in samples of heart muscle, meat, liver and kidneys of examined eagles are noteworthy. The most important finding within this research is the awareness of massive mercury contamination within the population of protected endangered wild birds. After entering the body, mercury inhibits enzyme systems, provokes lysosomal damage and poisoning of the central nervous system. The highest amounts of mercury are accumulated by the proteins of brain, liver, kidneys and gastro intestinal system *(Mihaljev et al., 2003)*. Mutagenic and teratogenic features and its well-established toxicity qualify mercury among the most dangerous environmental pollutants.

Conclusion

Results of our research indicated that samples obtained from wild birds revealed presence of toxic metals, above all the lead and mercury. Their highest average levels were determined in the liver and kidneys. Presence of detectable and measurable levels of cadmium and arsenic in all organs and tissues was surprising, and should be particularly emphasized. In that respect, further research should be focused on investigating causes and origin of these elements in the soil and plants. Our results suggest that pollution of the biosphere with chemical contaminants should be systematically monitored to identify potential increasing contamination tendencies and with an aim of producing healthy and safe food and environmental protection. Effective protection of wild birds against these dangerous agents requires comprehensive examination of largest possible number of samples in order to locate the pollution sources and obtain a more realistic picture of the investigated regions.

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Određivanje toksičnih elemenata kod divljih ptica sa područja Vojvodine

Ž. Mihaljev, M. Živkov-Baloš, M. Kapetanov, S. Jakšić

Rezime

Sadržaj olova, kadmijuma, arsena i žive ispitan je u 24 uzorka divljih ptica, i to: labud *(Cygnus)*, barska koka (*Gallinula choloropus)*, mala bela čaplja *(Egretta garzetta)*, orao mišar (**Buteo buteo)**, orao belorepan (*Haliaeetus albicilla)*, galeb (*Larus ridibundus*), bela roda (*Ciconia ciconia*), divlja patka (*Anas platyrhynchos*), siva čaplja (Ardea cinerea). Od sakupljenih ptica posebno je analizirano mišićno tkivo, jetra, bubrezi i srce. Uzorci za merenje su pripremljeni metodom vlažne digestije u sistemu Ethos, Labstation Microwave, Milestone. Olovo, kadmijum, arsen i živa određeni su metodom induktivno spregnute plazme na instrumentu Agilent ICP-MS 7700. Najveći sadržaj olova izmeren je u jetri i bubrezima ispitivanih ptica i iznosio je u proseku 0,372 ± 0,290 mg/kg za jetru i 0,384 ± 0,168 mg/kg za bubrege. U jetri i bubrezima ispitivanih ptica takođe je izmeren i najveći sadržaj kadmijuma, koji je prosečno iznosio u jetri 0,083 ± 0,077 mg/kg, a u bubrezima 0,248 ± 0,052 mg/kg. Utvrđeni sadržaj arsena u svim ispitanim uzorcima je veoma nizak (< 0,10 mg/kg). U nekim ispitanim uzorcima utvrđeno je prisustvo žive u veoma povišenim koncentracijama. U uzorku mesa sive čaplje koncentracija žive iznosila je 0,717 mg/kg, u jetri orla belorepana 1,128 mg/kg a u bubregu orla belorepana čak 3,656 mg/kg.

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STUDIES ON SOME SERUM ENZYMES IN TWO BULGARIAN INDIGENOUS SHEEP BREEDS

G. Angelov[1], I, Dimitrova[2], T. Mehmedov[1], P. Stamberov[1], N. Stancheva[3], S. Georgieva[4], G. Nakev[3]

[1]Faculty of Veterinary Medicine, University of Forestry, Sofia, Bulgaria
[2]Agronomy Faculty, University of Forestry, Sofia, Bulgaria
[3]Agricultural Institute – Shumen, Bulgaria
[4]Faculty of Agriculture, Trakia University, Stara Zagora, Bulgaria
Corresponding author: gen_angelov@mail.bg
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Abstract: The study included 140 clinically healthy animals from two indigenous sheep breeds - Karakachanian and Copper Red Shumen over one year old. The levels of serum ASAT, ALAT, Alkaline Phosphatase, KC (Creatine Kinase) and LDH (Lactate Dehydrogenase) were determined. The obtained results show that the values of ASAT and ALAT between the breeds are no significant differences, due to which these serum enzymes have smaller breed informational value. Levels of Alkaline Phosphatase, KC and LDH in Copper Red Shumen in varying degrees higher than the Karakachanian breed.

Key words: sheep, indigenous breeds, ASAT, ALAT, Creatine Kinase, Lactate Dehydrogenase

Introduction

Recent years, especially after accession of our country to the EU, sheep breeding in Bulgaria marks a positive development and has emerged as one of the priority sub-sectors in agriculture. All of this objective require in the veterinary clinical practice to expand and deepen our knowledge of some routine laboratory parameters examined in sheep. It is known that the level of serum liver enzymes in one degree or another, are among the most objective indicators of the overall condition of the body, enabling to evaluate the health status of animals. Therefore, their study represents an important source of information not only on the physiological state of the animals, but also to detect the earliest stages of the disease process (*Gurzhav et al, 1968; James, 1968; Kerr, 1989; Garry et al, 1990*). Information value of individual indicators among different species and breeds of animals is one of the prerequisites for proper evaluation and interpretation of the

results obtained (*Kaneko, et al., 1997; Bertoni, 1999; Kramer et al., 2000; Push, 2002*).

Many authors have studied the biochemical profile in sheep serum under normal conditions and in various diseases (*Boyd, 1988; Kramer, et al., 2000; Kerr, 2002*). Literature data on the normal range of a significant proportion of serum enzymes in sheep showed considerable variability which hinders their precise clinical interpretation (*Boyd, 1988; Kaneko et al., 1997; Kerr, 2002*). Too scarce and contradictory are the literature data on the activity of serum enzymes in Bulgarian local indigenous breeds whose populations in recent years has increased (*Binev et al., 2008; Todorova et al, 2010*). This requires their more comprehensive and thorough study and originated the present study.

Aim of this study was to perform a comparative study on some of the most frequently investigated laboratory practices serum enzymes in two local Bulgarian indigenous sheep breeds - Karakachanian and Copper Red Shumen, which would improve their informational value and clinical interpretation.

Material and Methods

Studies were conducted on 140 clinically healthy sheep of local breeds Copper Red Shumen (n=70) and Karakachanian (n=70) aged over one year, grown in the State Enterprise "Kabiuk" - Shumen. Four times, at intervals of one week was obtained blood from v. jugularis for determination of serum Alanine Aminotransferase (ALAT), Aspartate Aminotransferase (ASAT), Lactate Dehydrogenase (LDH), Creatine Kinase (CK) and Alkaline Phosphatase. Blood samples were taken in vakueti for serum with gel-clot activator and tested with ready kits “Human”, using a semi-automatic biochemical analyzer Screen master LIHD-113, manufactured by the German company Hospital Diagnostic. All results obtained were processed statistically with variance-software program Anova.

Results and Discussion

The obtained results of the studies are presented in Table. 1 and show some differences in the level of studied serum enzymes between the two sheep breeds.

Average values of ASAT levels not reliably indicate significant breed differences. In Copper Red Shumen established average of 127.41 ± 37.39 U/l, which is approximately 5% higher than those of Karakachan breed – 121.29 ± 32.34 U/l. A similar trend was found in the level of ALAT, the activity of which in Copper Red Shumen (27.91 ± 9.49 U/l) is approximately 10% higher than that of Karakachanian (25.2 l ± 6,51 U/l). Like ASAT and ALAT levels of Alkaline Phosphatase repeated but expressed at greater degree differences between the two

breeds. For Copper Red Shumen values of 227.2 ± 32.96 U/l were established, which is 17% higher than those of Karakachanian breed (192.9 ± 36.50 U/l). Reliably significant differences between the two breeds are found in serum LDH and CK.

Table 1. Mean parameters of some serum enzymes in indigenous sheep breeds

Parameters	BREED	
	Copper Red Shumen	Karakachanian
	n = 70	n = 70
ASAT U/l	127,4157 ±37,3929	121,2944 ±32,3459
ALAT U/l	27,9128 ±9,4955	25,2746 ±6,5144
LDH U/l	1343,599 ±80,3007	759,0871 ±61,5697
Creatine Kinase U/l	346,5194 ±53,0889	287,2408 ±50,8578
Alkaline Phosphatase U/l	227,2786 ±32,9636	192,9408 ±36,5091

Values obtained from 346.5 ± 53.08 U/l for CK in Copper Red Shumen are 20% higher than those of Karakachanian breed (287.2 ± 50.85 U/l).

The most significant breed differences were found in serum LDH. Values established in Copper Red Shumen (1343.5 ± 80.3 U/l) were approximately two times higher than those obtained in Karakachanian breed (759.0 ± 61.56 U/l).

Analysis of obtained results shows that the Copper Red Shumen breed values of ASAT, ALAT, Alkaline Phosphatase, CK and LDH in different degrees were higher than the same of Karakachanian breed. Regardless of these differences found in our results for ASAT, ALAT, Alkaline Phosphatase are in the normal range for the species and correlate with those cited by other authors (*Kaneko et al., 1997; Kerr, 2002; Push, 2002; Todorova, et al, 2010*). Other authors (*James, 1968; Gurzhav et al, 1968; Binev et al., 2008*) report obtained significantly lower values for ASAT compared with our research. These differences are probably related as belonging to different breeds of test animals as well as the age, sex and seasonal differences in the conduct of these studies. Our results of the activity of CK and LDH are significantly higher than those of the *Push (2002)*, but correlated with those of *Todorova* et al, 2010, who studied the CK in sheep from breeds Cigai and Karakachanian. The creatine kinase as an enzyme catalyzing the transfer of macro-power phosphate linkages of ATP on muscle creatine and the lactate dehydrogenase as oxidoreductase, catalyzing the two-way transition from aerobic to anaerobic glycolysis (lactate ↔ pyruvate) are considered as enzymes, reflective

the functional activity of the muscle tissue. Meat productive direction of Copper Red Shumen breed determines undoubtedly considerable variation in the intensity of metabolic processes, in particular in muscle tissue, which is completely correlated with the obtained higher values of CK and LDH in comparison with Karakachanian breed.

Conclusion

There are no breed differences in the level of ASAT and ALAT between sheep breeds Copper Red Shumen and Karakachanian.

In Copper Red Shumen breed activity of creatine kinase and lactate dehydrogenase were higher than in Karakachan breed.

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Ispitivanje enzima seruma dve bugarske autohtone rase ovaca

G. Angelov, I. Dimitrova, T. Mehmedov, P. Stamberovв, N. Stancheva, S. Georgieva, G. Nakev

Rezime

U istraživanje je bilo uključeno 140 klinički zdravih životinja dve autohtone rase ovaca – karakačanske i bakarno crveni šumen, starosti više od jedne godine. Određivani su nivoi ASAT, ALAT, alkalne fosfataze, KC (kreatin kineaze-Creatine Kinase) i LDH (laktat dehidrogenaze-Lactate Dehydrogenase) u serumu. Dobijeni rezultati pokazuju da postoje značajne razlike u vrednostima ASAT i ALAT među rasama, zbog kojih ovi enzimi imaju manju informativnu vrednost za rasu. Nivoi alkalne fosfataze, LDH i CK u bakarnoj crvenoj šumen rasi su u različitim stepenima bili viši od nivoa utvđenih kod grla karakačnske rase.

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BIOLOGICAL EFFECT ESTABLISHMENT OF *JODIS CONCENTRATE* SUPPLEMENTATION IN RABBIT DOES

S. Grigorova, M. Petkova, Kr. Velikov

Institute of Animal Science Kostinbrod 2232, Bulgaria
Corresponding authors: m_petkova2002@abv.bg; svet.grigorova@abv.bg
Original scientific paper

Abstract: The objective of this study was to examine the effect of iodine from *Jodis concentrate* produced in Ukraine in dose 0.2ml/l drinking water for different categories rabbits. An experiment was carried out with 2x7 nulliparous rabbit does (first stage) (4.5-5 months old from White New Zealand breed) and their born offspring (second stage). The does were randomly distributed in two groups: control and experimental. All animals were fed with one and the same granulated Total Mixed Ration for parents. All rabbit does were mated by the same buck from Californian breed for a period of 5 days. During the second stage of the study we observed growth and development of litter size from both groups' rabbit does. Three groups from weaned rabbits were formed, 20 animals per each (10 male and 10 female): control (without additive) and Ist experimental group (with *Jodis*) - from rabbit does without *Jodis*; IInd experimental group (with *Jodis*) – from treated rabbit does. We concluded that *Jodis concentrate* is suitable source of iodine for both rabbits does and newborn rabbits. The following more important effects of additive we found in rabbits does: the pregnancy duration was significantly decreased ($P<0.001$); the total born rabbits and the average litter size had a tendency to increase. The results obtained showed that male and female rabbits from both experimental groups had higher average daily gain. Male rabbits of the I experimental group had highest average daily gain (15% higher than control) and with significantly lower feed conversion ratio ($P< 0.05$).

Key words: *Jodis concentrate,* female rabbits, White New Zealand breed, biological effects.

Introduction

Iodine is an indispensable trace element that becomes a raw material of the thyroid hormone for promoting metabolism of a living body. Iodine enters the body of humans and animals through foods and feeds, water and air (coastal areas). According to *Kaufmann et al. (1998)* iodine deficiency is one of the four most

severe types of malnutrition worldwide (in addition to protein-energy malnutrition, nutritional anemia and Vitamin A deficiency). Iodine deficiency can lead to serious diseases not only in humans but also in animals. The majority of farm animal feeds is plant matter which usually contains little iodine (< 20 – 50 µg/kg DM) and the iodine content of water is generally low (2-7 µg/l) (*Schöne and Rajkumar Rajendram, 2009*) . Reduced iodine content in the feed and water used by animals rapidly decreases the immune system on one side and the reduction in the animal's body from other (*Gabrashanski et al., 1979*). Iodine deficiency leads to thyroid hypo function, causing multiple interferences in the physiological functions and biological processes. These results in the reduction of fertility, an increase in the number of abortion, suppress the growth of young animals. The objective of an optimum supply of iodine to farm animals is motivated first of all by an effort to reduce losses caused by clinical forms of its deficiency, significantly influences iodine content in animal products – meat, milk and eggs (*Travnicek et al., 2006*). The most usual way for supplying iodine in both food and feed is using salt enriched with iodine (iodinated salt). But depending of time of storage the iodine from this salt can be lost. The average one goes up to 50% (*Gjorgovska and Filev, 2010*).

Requirements of animals of iodine depend on its type, breed, physiological status and season. Pregnant animals and young organisms need more iodine. Animal nutrition societies recommended iodine supplements of 120-250 µg/kg DM – for growing pigs and beef cattle. This prevents iodine deficiency, facilitates high performance. Adequate iodine nutrition is 100-299 µg/l. There is insufficient observation on the biological effects of the addition of iodine in rabbits in the available literature. *Marinov et al. (2006)* summaries three different authors and recommends iodine level in rabbits TMR 0.2 mg/kg feed.

Jodis concentrate, which is a new and more stabile source of iodine, had been exanimate by poultry, pigs, cattle (lactating cows and calves) and horses (www.jodis-k.com). But, there are no any experiments on rabbits. That was why the aim of our investigation was to examine of biological effect of iodine in biological active form from *Jodis concentrate* produced in Ukraine.

Material and Methods

The tested additive *Jodis concentrate* is produced in Ukraine. It is mineral water with high quality supplemented by special technology with biologically active iodine. *Jodis concentrate* contains : 80 mg/dm^3 iodine in biological active form, 10-100 mg/dm^3 /Na + K , 5-150 mg/dm^3 Ca, 10-100 mg/dm^3 Mn, 50 mg/dm^3 chlorides, 50 mg/dm^3 sulphates and 300-600 mg/dm^3 hydrocarbons. The product

has no any small, taste and color. The product is harmless. There were no side effects (*www.jodiscentr-bg.com/index).*

The present study was carried out in the period April-June at the Experimental base of the Institute of Animal Science Kostinbrod with a total of 2x7 nulliparous rabbit does (White New Zealand breed) at the age of 4.5-5 months with an initial average live weight 3.630kg. The animals were randomly divided in two groups – a control and experimental and raised individual in one floor cage complex. Rabbit does from the both groups were mated with one and the same buck from Californian breed for a period of 5 days. The both groups received the same granulated Total Mixed Ration (TMR) for parents (Table 1). The nutritive value of the diet was determined by conventional Weende method. The rabbits does were fed restrictively during the first week after mating but from the second week - *ad libitum*. Water was supplied via nipple watering trough. The additive *Jodis concentrate* was supplemented to the drinking water of experimental group in dose 0.2 ml/l water. Feed intake (once weekly), amount of drinking water with *Jodis concentrate* in ml (daily), litter size, weight of the litter size on the 15^{th} , 21^{st} , 30^{th} , 45^{th} *post partum* and weight of young rabbits at the weaning (g) were controlled. Vitality, development and mortality of newborn rabbits were observed daily.

Table 1. Ingredients and chemical composition of TMR for rabbits' parents

Ingredients	%
Maize	7.95
Barley	20.00
Soybean meal, solvent 46% CP	8.00
Sunflower meal, solvent 34% CP	18.00
Wheat bran	15.00
Alfalfa meal	25.00
Wheat straw	3.00
Salt	0.40
Limestone	1.50
D-C-P (Dicalcium Phosphate)	0.50
L-Lysine	0.15
Vitamin and Mineral Premix Guyombalk 6642	0.50
TOTAL	100.00
Chemical composition	%
Digestible Energy, Kcal/kg	1839.00
Moisture	10.80
Crude protein	19.30
Crude fiber	14.20
Crude fat	2.50
Ca, total	1.17
P, total	0.64
Lysine	0.84
Methionine + Cystine	0.65

During the second stage of this study we observed further growth and development of weaned rabbits from both groups' rabbit does. Three groups from weaned rabbits were formed, 20 animals per each (10 male and 10 female): control (without additive) and I experimental group (with *Jodis*) – from control rabbit does without *Jodis*; II experimental group (with *Jodis*) – from rabbit does received additive. The dose of *Jodis concentrate* for the growing rabbits from both experimental groups was 2 ml/l drinking water. The animals were raised in wire cages, lined in a single layer, 5 rabbits in each. The trial lasted 48 days. All rabbits received *ad libitum* standard granulated TMR for growing rabbits with the next nutritional characteristic: 16.9% crude protein, 13.2% crude fiber, 3% crude fat, 0.96% Ca, 0.64% P. Water was supplied via nipple watering trough. Following parameters were controlled: daily amounts of drinking water with *Jodis concentrate* (ml); body weight and feed intake (g) –once weekly. Vitality and mortality in all rabbits were observed daily.

All data are presented as means with their standard errors. Statistical examination of obtained results was determined by Excel 2000, single factor, ANOVA program.

Results and Discussion

The average consumption of *Jodis concentrate* of experimental rabbit does was 0.94 ml. During the period birth - weaning they received together with rabbits in box average 1.96 ml/doe/day *Jodis concentrate*.

Table 2. Results from first experimental stage with rabbit does (x ± SE)

Items	Control group n=7	Experimental group n=7
Duration of pregnancy, days	30.28±0.46***	32.29±0.29***
Total born	53	62
Litter size	7.57±0.84	8.96±1.35
Weaned rabbits, total	37	36
Number of weaned rabbits/doe	5.29±1.12	5.14±1.22
Feed intake by mating up to birth/ doe, g	5632±206.77	5160±175.57
Feed intake/doe + rabbits/day, g	177.39±5.12	176.03±4.82
Mortality of newborn rabbits to the second day *post partum* , %	18.87	24.19
Mortality of young rabbits from 3rd until 21st days *post partum*, %	3.77	4.84
Mortality of young rabbits from 22nd day *post partum* up to weaning, %	7.55	12.90
Mortality of young rabbits throughout the experiment, %	30.19	41.94

Significant: ** * - P< 0.001

The obtained experimental results for the rabbit does are presented in Table 2. Pregnancy duration for treated does was significant lower in compare to the control group (P<0.001). This fact may be explained by the established negative correlation between litter size and pregnancy duration in young does *(Marinov et al., 2006*). All newborn rabbits were well formed. Total litter size of the treated does was 17% higher than control group. Average litter size also was higher (18%). The minimum number of litter size in the control group was 3 and its maximum – 10.This items for experimental does were 4 and 14 respectively. One doe per each group lost their newborn rabbits. That is why they were eliminated from the trial as a whole. The higher mortality (11%) observed in the experimental group may be explained by the limited ability of rabbit does to raise higher number of young rabbits. Therefore, the number of weaned rabbits in the both groups was almost the same (37 in control group and 36 in experimental group) regardless of the higher fertility in the treated group. These data are well corresponded with the data of decline in weight growth of experimental young rabbits (Table 3).

Table 3. Rabbits` growth dynamic (n=2x6) and feed consumption between birth and weaning (x ± SE)

Items	Control group	Experimental group
Litter weight, g: on the 15th day *post partum*g	268,34±25,14	229.46±19,74
on the 21st *post partum*	377.83±37.75	308.1±25.34
on the 30th *post partum*	713.55±58.63	593.09±40.72
on the 45th *post partum*	1234±80.38	1085.04±45.25
at weaning	1367±62.58	1241.84±121.84
Average daily feed consumption of does + rabbits for a period birth-weaning, g	510.33±30.93	454.32±40.98
Length of the period birth-weaning, days	48±0.82	46.33±0.56

The results have a tendency towards to increase in experimental group. The same trend followed the results for feed consumption during the period birth-weaning. This reflected on the decreased by 2 days of the average duration for the period birth - weaning in the treated animals. The development of young rabbits (grows a coat, eyes opening, feed supply) from the both groups proceed normal.

During the growth experiment the rabbits from the treated groups received following daily amounts of *Jodis concentrate*: Iexperimental group - 0.46 and 0.56 ml for female and male animals respectively, II experimental group – 0.50 ml for female and 0.43 ml for male rabbits (Table 4).

Table 4. *Jodis concentrate* intake during the second experimental stage with growth rabbits, (x ± SE)

Groups / Parameters	I experimental group		II experimental group	
	♀ n=10	♂ n=10	♀ n=10	♂ n=10
J concentrate/head/day, ml	0.46±0.04	0.53±0.02	0.50±0.04	0.43±0.04

The results obtained showed that male and female rabbits from both experimental groups had higher average daily gain than these of control group (Table 5).

Table 5. Rabbits` growth parameters, (x ± SE)

Groups / Parameters	Control		I experimental group		II experimental group	
	♀	♂	♀	♂	♀	♂
n	10	10	10	10	10	10
Period, days	48	48	48	48	48	48
Initial weight, g	1457±52.42	1544±66.44	1474±23.61	1416±61.24	1443±59.37	1564±55.42
Final weight, g	2719±73.55	2758±68.56	2848±78.29	2814±67.84	2825±73.01	2855±84.70
ADG, g/day	26.29±1.20	25.28±1.44	28.63±1.11	29.11±1.52	28.80±0.69	26.89±1.62
%	100	100	108.9	115.15	109.5	106.4
Feed intake, g//head/day	113.11±4.59	115.13±5.76	116.71±6.90	115.51±7.12	117.46±7.22	118.38±5.68
FCR, g/kg	4302±194	4552±217 a	4077±227	3966±134 a	4080±240	4401±234
%	100	100	94.8	87.1	94.8	96.7
Mortality	-	-	1	-	-	1

Significant: a - * - P< 0.05

Male rabbits from the first experimental group had highest average daily gain (15% higher than control) and their feed conversion ratio was significantly lower (P<0.05). These rabbits had a lower body weight than the other groups at the

beginning of the experiment, independently of the fact, by the end of the trial they compensate for the delay in growth up to weaning. This result confirms the findings of *Kowalski* that the addition of iodine increases the adaptive capacity of the organism and improves metabolism, growth and utilization of the nutritive substances in the feed (*Gabrashanski et al., 1979).*

Conclusion

Jodis concentrate is suitable source of iodine for both categories rabbits - does and newborn rabbits.

- The average intake of *Jodis concentrate* was 0.94ml/day/rabbit does;
- Pregnancy duration was significant lower by additive ($P<0.001$).
- Total born in the experimental group as well as average litter size was higher than the control group 17% and 18% respectively.
- The average intake of *Jodis concentrate* from rabbits at both experimental groups was 0.51 ml (I experimental group) and 0.46 ml (II^{nd} experimental group).
- All rabbits (male and female) from both experimental groups had higher average daily gain than the control group.
- *Jodis concentrate* increases the adaptive ability of the organism: male rabbits from I^{st} experimental group had highest average daily gain (15% higher than control) and feed conversion ratio was significantly lower ($P<0.05$).

Acknowledgment

We would like to thank to Mr Manol Nikolov from "STEFI – S. Nikolova" Ltd, Razgrad, Bulgaria for providing the product *Jodis Concentrate* used in our study.

Biološki efekat korišćenja *Jodis* koncentrata u ishrani kunića

S Grigorova, M. Petkova, Kr. Velikov

Rezime

Cilj ovog istraživanja bio je da se ispita uticaj joda iz preparata *Jodis koncentrate* proizvedenog u Ukrajini u dozi od 0.2ml/l vode za piće za različite

kategorije kunića. Ogled je izveden na 2x7 kunića (prva faza) (uzrasta od 4.5-5 meseci, bele novozelandske rase) i njihovom potomstvu (druga faza). Grla su nasumično raspoređena u dve grupe: kontrolna i eksperimentalna. Sve životinje su hranjene istim granulisanim kompletnim mešovitim obrokom za roditelje. Sve ženke su parene istim mužjakom kalifornijske rase u trajanju od 5 dana. U drugoj fazi studije pratili smo rast i razvoj veličine legla obe grupa zečeva. Tri grupe su formirane od odlučenih zečeva, 20 životinja po grupi (10 mužjaka i 10 ženki): kontrola (bez aditiva) i I eksperimentalna grupa (sa *Jodisom*) – sastavljena od ženki zečeva bez *Jodisa*; II eksperimentalna grupa (sa *Jodisom*) - sastavljena od tretiranih ženki zečeva. Zaključeno je da je *Jodis* koncentrat pogodan izvor joda i za ženke i novorođene zečeve. Sledeći važniji efekti aditiva koje smo pronašli kod ženki su: trajanje trudnoće je znatno smanjeno ($p<0,001$), broj ukupno rođenih zečeva i prosečna veličina legla imali su tendenciju porasta. Dobijeni rezultati su pokazali da su mužjaci i ženke zečevi iz obe ogledne grupe imali veći prosečni dnevni prirast. Mužjaci u I eksperimentalnoj grupi su imali najveći prosečni dnevni prirast (15% veći od kontrole) i sa znatno manjom konverzijom hrane ($P <0,05$).

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INFLUENCE OF MATING UNTIL 48-TH HOUR POST WEANING ON THE REPRODUCTIVE PARAMETERS IN WHITE NEW ZEALAND RABBIT DOES

K. Velikov

Institute of Animal Science – Kostinbrod, Post code: 2232, Kostinbrod, Bulgaria
Corresponding author: sherry_bg@abv.bg
Original scientific paper

Abstract: The aim of present experiment was to examine the influence of weaning of litter on some of the reproductive parameters in the next reproductive cycle. The study was conducted in the experimental farm of the Institute of Animal Science – Kostinbrod, Bulgaria. Were examined 63 reproductive cycles - control group (25) and experimental group (38). The rabbits were fed restrictively with commercial pellets and watered *ad libidum* with nipple drinkers. The does of experimental group were moved in cage of buck on the 4-th, 24-th and 48-th hour until mating was done. The mating of does, until day 2 post litter weaning, increased accepting rate (+12.77%), the number of total born (+15.82 %), the number of born alive (+22.51 %), weight of total born (+2.98%) and weight of born alive (+12.33%) in the next reproductive cycle. Decrease of parameters was observed in fertility rate (-10.76%), average weight of rabbits in total born litter (-9.71%) and average weight of born alive rabbit (- 9.15%).

Keywords: mating, rabbit, reproductive parameters, weaning, White New Zealand.

Introduction

As a multifetal animal, the reproductive parameters are essential for the effectiveness of rabbit breeding, so they have to be permanently controlled. Increasing those parameters, we could raise the rabbit productivity.

Most commonly, intensive and semi-intensive reproductive rhythm are used in professional rabbit farms. Usually, insemination of does is done until day 11 *post partum.* There is an antagonism between reproduction and lactation. To be overcome it, hormonal therapies are used in professional rabbit farms. The European member prefers to use an environmental meat for consumption, because of this many rabbit scientists worked on the problem of improving reproductive parameters without any exogenous substances. There were examined many

environmental methods (light stimulation, dam-litter separation, flushing, change of cages etc.), usually borrowed by other species. Those methods were named biostimulations (*Theau-Clement et al., 2006*).

Dimitrova et al. (2009) compared two schemes of hormonal stimulation. First scheme included application of PMSG and GnRH. In the second it was applied only GnRH. As a result in first case they obtained 28.57% fertility rate and in second – 62.50% fertility rate. White New Zealand does showed higher level of fertility then Californian does. Such a higher level of fertility was observed by *Dimitrova et al. (2010).*

In Bulgaria, the majority of rabbits are bred in small family farms (*Dimitrova et al., 2008*). There, usually, the does are mated some days post weaning of previous litter. Because of this, the aim of present paper was to examine the influence of weaning of litter on some of the reproductive parameters in the next reproductive cycle.

Material and methods

The experiment was conducted in the experimental rabbit farm of the Institute of Animal Science - Kostinbrod between the March and the July 2012. 63 reproductive cycles were examined - control group (25) and experimental group (38). The does and bucks were bred in individual cages. The size of cage was 800/600/350 mm. The rabbits were watered *ad libidum* through nipple drinkers. The bucks were fed with 200 g commercial pellets for fattening, and does – with commercial pellets for does. From mating to birth, the does were fed with 200 g, from the birth to the day 16 *post partum* – 300 g, and from the day 17 *post partum* to the weaning of litters – 400 g pellets. The litters were weaned between day 35 and day 40 *post partum*.

The does of experimental group were placed in the buck's cage after the 4-th, the 24-th and between the 46-th and 48-th hour post litter weaning until they were mated. The does, which did not be mated until 48-th hour, left the experiment. In the does of control group were placed in the buck's cage at least 72 hours post litter weaning.

The dates were processed statistically with MS Excel 2003.

Results and Discussion

Accepting rate (AR). The accepting rate of experimental group was 94.73%, and those of the control group – 84.00%. It shows that accepting rate is higher with 12.77%, when does are mated until the 72-nd hour.(Table 1, Figure 1)

Table 1. Accepting rate, fertility rate and percentage of fertilized does in the groups, %

Parameters	Control group (100%)	Experimental group	Differences (±%)
Accepting rate	84,00	94,73	+ 12.77
Fertility rate	80,93	72,22	- 10.76
Fertilized does in groups	67.98	68.41	+0.63

Fertility rate (FR). Fertility rate in the experimental group was lower from the control group with 10.76%. The fertility rate in the experimental group was 72.22%, and in the control group – 80.93 % (Table 1, Figure 1).

Percentage of fertilized does in the groups (FD). We use this parameter to create a clearer picture of the effectiveness of mating in does until the 48-th hour post weaning. It was calculated by the formula:

$$FD = \frac{AR \times FR}{100}$$

FD – Fertilized does in the groups (%)

AR – Accepting rate
FR – Fertility rate

The values of this parameter in both groups are very similar (Fig.1). In the experimental group it was 68.41%, and in the control group – 67.98% (Table 1).

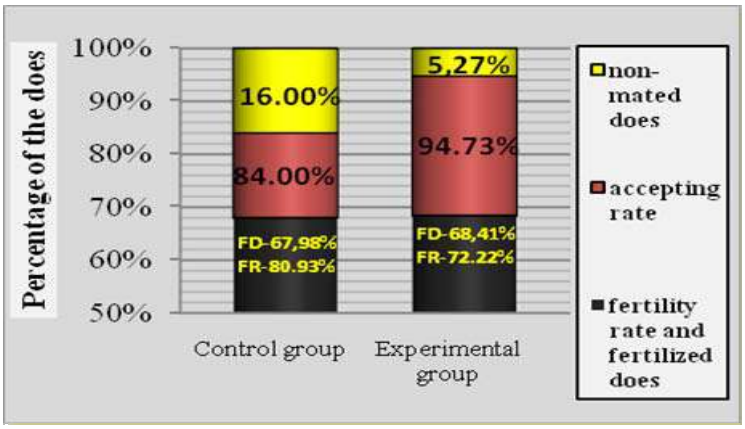

Figure 1. Accepting rate, fertility rate (FR) and percentage of fertilized does (FD)

Total born. The litter size in the experimental group was 15.82% higher than the control group. It was 9.81 in the experimental and 8.47 in the control group and the differences was significant (P<0.05, Table 2, Figure 2)

Number of born alive. The number of born alive rabbits in the experimental group was 22.51% higher than the control group. In the experimental group, it was 9.58, and in the control group – 7.82 (P<0.01, Table 2, Figure 2).

Weight of total born rabbits. The weight of total born litter in the experimental group was 2.98% higher than that of the control group, but the differences were not significant (P>0.5). In the experimental group it was 570.39 g, and in the control group – 553.88 g, (Table 2, Figure 3).

Table 2. Duration of pregnancy and litter parameters in birth

Parameters	Control group (100%)	Experimental group	Differences (±%)	Signifi-cance
Litter size, number	8,47±2,32	9,81±2,15	+ 15.82	*
Born alive,number	7,82±2,21	9,58±2,28	+ 22.51	**
Weight of total born, g	553,88±96,34	570,39±93,95	+2.98	NS
Weight of born alive, g	497,18±104,92	558,50±96,28	+ 12.33	*
Average weight of total born rabbits, g	65,91±13,47	59,51±9,47	- 9.71	NS
Average weight of born alive rabbits, g	65,99±13,60	59,95±9,77	- 9.15	NS
Duration of pregnancy, days	31,47±0,72	31,38±0,98	-0.29	NS

NS – non-significant, * - P<0.05, ** - P<0.01

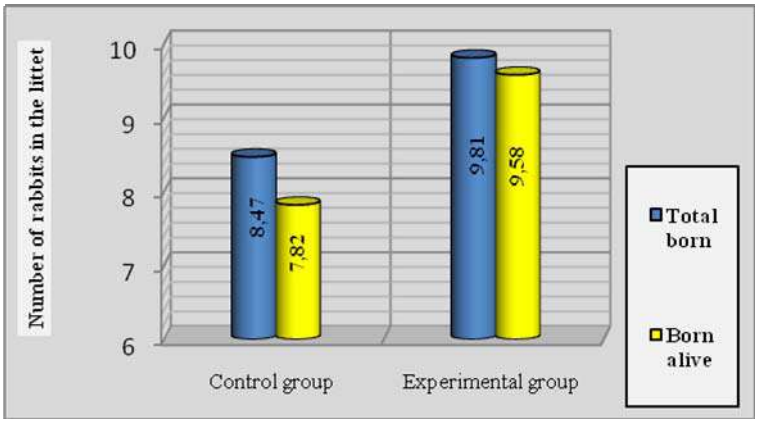

Figure 2. Number of rabbits in the litter

Weight of born alive rabbits. The weight of born alive litter was 12.33% higher in the experimental group than in the control group. In the experimental group it was 558.5 g, and in the control one – 497.18 g. The differences were significant (P<0.5, Table 2, Figure 3).

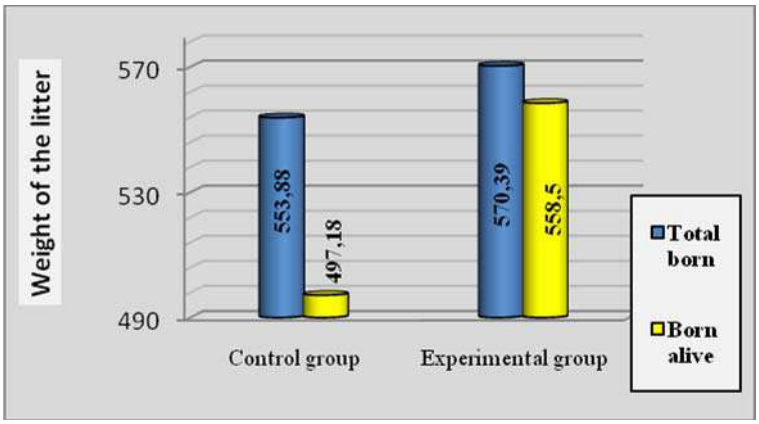

Figure 3. Weight of the litter (g)

Average weight of total born rabbits. The average weight of total born rabbits in the control group was higher than the experimental one with 9.71%. In the control group it was 65.91 g, and in the experimental group – 59.51g (P>0.05). Probably, this is due to higher values of litter size in the experimental group (Table 2, Figure 4).

Average weight of born alive rabbits. The values of this parameter are similar to those of previous one. In the experimental group it was 59.95 g, and in

the control group – 65.99g. The differences are not significant, too (P>0.05, Table 2, Figure 4).

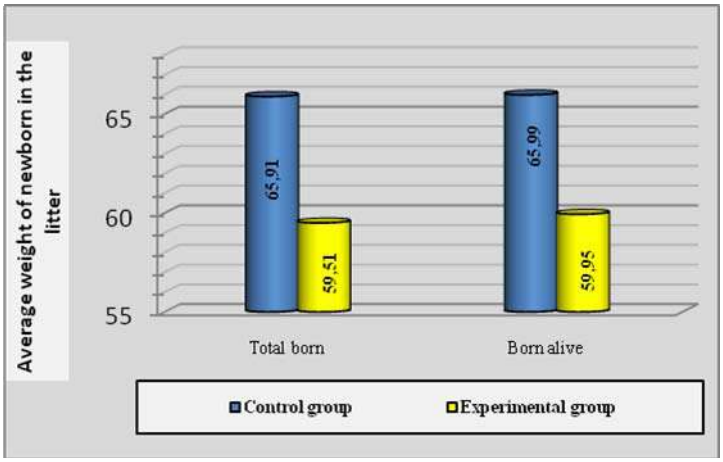

Figure 4. Average weight of rabbit in the litter (g)

Duration of pregnancy. The duration of pregnancy is a relatively constant parameter. It depends on many factors (litter size, the age of does and bucks etc.). The duration of pregnancy in the control group was 31.47 days, and in the experimental one – 31.38 days. Probably, the higher values of duration of pregnancy in the control group is due to its lower values of litter size (Table 2).

The mating until the 48-th hour post weaning increased accepting rate, but decreased fertility rate, so the percentage of fertilized does was not changed at all. The statistical significant differences were observed at some of the litter parameters. Based on experimental dates, we can assert, that the mating until the 48-th hour increases the total born and born alive rabbits. The weight of newborn rabbits were not influenced at all, except the weight of born alive rabbits. It was increased in the experimental group, probably due to higher stillborn rabbits. The duration of pregnancy, as a relatively constant parameter, did not show any big differences.

Conclusion

The mating until the 48-th hour after weaning of previous litters could be used as a method of increasing of some reproductive parameters in an extensive breeding cycle (litter size, weight of born alive litter) and for synchronization of the

estrus in White New Zealand does. In the present experiment, influence on the average weight of newborns in the litter was not observed.

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Uticaj parenja do 48 sati nakon odlučivanja na reproduktivne parametare ženskih kunića bele novozelandske rase

K. Velikov

Rezime

Za efikasnost uzgoja kunića, kao multipare životinje, reproduktivni parametri su od suštinskog značaja. Najčešće, na profesionalnim farmama, koristi se intenzivni i poluintenzivni reproduktivni ritam. U Bugarskoj, većina zečeva se gaji na malim porodičnim gazdinstvima. Obično se ženke pare nekoliko dana posle odbijanja od prethodnog legla.

Cilj ovog rada bio je da se ispita uticaj odbijanja legla na neke od reproduktivnih parametara u narednom reproduktivnom ciklusu. Istraživanje je sprovedeno na eksperimentalnoj farmi Instituta za stočarstvo - Kostinbrod, Bugarska, između marta i jula 2012. Statistički značajne razlike su uočene kod nekih parametara legla. Na osnovu eksperimentalnih podataka, možemo tvrditi, da parenje do 48 sati posle odbijanja povećava broj ukupno rođenih i živorođenih kunića. Masa novorođenih zečeva nije bila pod uticajem, osim mase živorođenih kunića. Parenje do 48 sati posle odbijanja utiče na povećanje stope prihvatanja, ali i na smanjenje stope plodnosti, tako da je procenat oplođenih ženki ostao nepromenjen.

Parenje do 48 sati po odbijanju od prethodnih legla može da se koristi kao metod poboljšanja nekih reproduktivnih parametara u ekstenzivnom uzgoju (veličine legla, težine živorođenih kunića, odnosno legla) i za sinhronizaciju estrusa ženki novozelandskih belih kunića.

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FUNCTIONAL ACTIVITY OF THE ADRENAL GLANDS, RECTAL TEMPERATURE AND SOME HEMATOLOGICAL INDICES IN RABBITS REARED UNDER LOW AND HIGH INDOOR AMMONIA LEVELS

M. Dyavolova, D. Gudev, I. Yanchev, P. Moneva

Institute of Animal Science, 2232 Kostinbrod, Bulgaria
Corresponding author: m.dyavolova@gmail.com
Original scientific paper

Abstract: The object of the present experiment was to evaluate the effect of ammonia on the adrenal response to stress, heterophil to lymphocyte ratio, hematocrit, number of erythrocytes, number of leukocytes and rectal temperature in rabbits. Twelve New Zealand White male rabbits (mean age 4 months, and average weight 3 kg) were allocated into two groups, raised in wire cages. The rabbits in the first group, unlike those in the second group, were raised at lower level of stocking density. The experiments were conducted in winter-spring (February - March - April) and summer (July) seasons. Air ammonia levels in the room having higher stocking density were within 22.1 – 42.7 ppm in winter-spring season and 2.1 – 13.1 ppm in the summer, whereas ammonia levels in the room having low stocking density were significantly lower (2.1 – 13.1 ppm) in winter-spring season and in summer season (0.9 – 1.2 ppm). The rabbits were subjected to 1 min audio stress (dog barking) in a triple 110 dB playback. Dog barking did not cause significant elevation of plasma cortisol levels. Exposure to high level of ammonia resulted in decline in basal ($P<0.01$) and stress-induced ($P>0.05$) levels of cortisol as compared to the corresponding cortisol values in the rabbits raised under low air ammonia levels (spring experiment). Stress resulted in slight increase in heterophil to lymphocyte ratio which was more pronounced in the rabbits raised under lower ammonia levels ($P>0.05$). The rabbits raised under high ammonia levels had higher rectal temperature in summer ($P<0.01$) and winter ($P>0.05$). Hematocrit values were higher in winter than in summer ($P<0.01$). Total leukocyte and erythrocyte counts were not influenced by season and ammonia levels. This study suggests that long term exposure to high ammonia concentration inhibits adrenal function and affects thermoregulation in rabbits.

Key words: ammonia, cortisol, rectal temperature, hematological indices

Introduction

Ammonia is integral component of normal metabolic processes and plays an essential role in the physiology of animals. However, high ammonia levels can have detrimental effects on animal health and performance.

Ammonia is colorless, irritating gas that is normally produced in rabbit housing buildings. Ammonia is known to cause intense airway inflammation in acute exposure, but chronic exposure leads to hematological and functional changes. The available literature provides little and contradictory information about the adrenal response of rabbits to stress factors *(Szeto et al., 2004; Rovirosa et al., 2005)* responsible for stress reaction in rabbits.

Ammonia has been found to have either stimulatory *(von Borell et al., 2007)* or no effect *(Gustin et al., 1994)* on adrenal activity in pigs.

There is no available literature of how air ammonia, which is produced during the indoor housing of rabbits, affects hypothalamic-pituitary-adrenal axis.

There is scarce data about the effect of air ammonia on hematological indices such as hematocrit, heterophil to lymphocyte ratio, white blood count and erythrocyte count *(von Borell et al., 2007)*. However, we did not find any trials assessing hematological parameters in rabbits exposed to ammonia.

Consequently, the aim of this study was to evaluate the effect of aerial ammonia and season on hematological responses to stress in growing rabbits.

Material and methods

The experiment comprised 12 New Zealand White male rabbits (*Oryctolagus cuniculus*) at the age of 4 months, divided in two groups (control and experimental), consisting of 6 rabbits each. Rabbits in the control group were reared under low air ammonia concentration, and those from the experimental group were reared under high air ammonia concentration. The experiment was conducted in spring, summer and winter seasons. Average values of aerial temperature in the building were as follows: 9°C in spring, 24°C in summer and 4 °C in winter. Control rabbits were reared in rooms with low levels of aerial ammonia concentrations (2.1-13.1 ppm in spring; 2.1-13.1 ppm in winter and 0.9-1.2 ppm in summer). Experimental rabbits were reared under high ammonia levels (22.1-42.7 ppm in spring; 22.1-42.7 ppm in winter and 3.0-8.9 ppm in summer).

Rabbits were housed individually in wire-floor cages, provided with feeders and automated drinkers – feed and drinking water were supplied *ad libitum*.

Indoor air temperature and humidity were recorded througout the experiment by the psychrometer method. Stress was induced by 1 minute “dog barking” PC record in a triple 110 dB playback. Blood samples were taken before

and 25 minutes following the start of the stress episode. Rectal temperature was measured by digital thermometer immediately after second blood sampling procedure. Total erythrocyte and leukocyte count was determined by manual haemacytometer chamber count. Haematocrit was measured by the microhaematocrit method. Peripheral blood leukocytes were counted on smears, that were prepared immediately after the blood collection. The smears were stained using May-Grunvald and Gisma stains *(Lucas and Jamroz, 1961)*. Four hundred leukocytes including neutrophils, eosinophils, basophils, lymphocytes and monocytes were counted microscopically on a slide.Plasma cortisol was assessed by ELISA method – BioSource Rabbit Cortisol ELISA kit, following extraction of plasma with absolute ethanol. Air ammonia was recorded via AeroQual S200 Monitor, equipted with ammonia sensor head (0-100±0.1 ppm).

The results of one factor statistical analysis are expressed as means ±S.E.M. and were analyzed by ANOVA.

Results and discussion

Basal plasma cortisol level was significantly lower ($P<0.01$) in the rabbits reared under high aerial ammonia concentrations as compared to control animals (Figure 1). Exposure to psychological stress caused slight increase ($P>0.05$) of plasma cortisol in both groups.

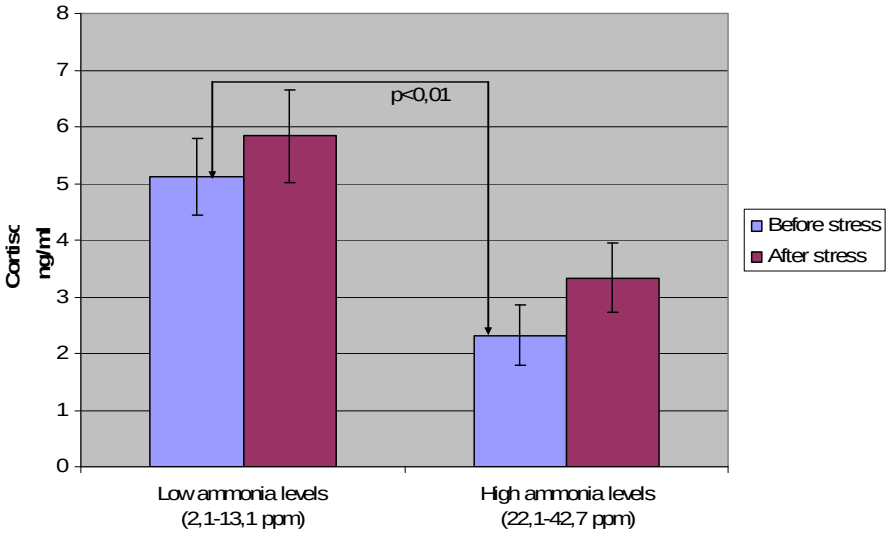

Fig. 1: Plasma cortisol levels and response to stress in rabbits, reared under low and high indoor air ammonia levels (spring values)

Exposure to ammonia at concentrations of 25 and 50 ppm for 14 days has been demonstrated to have no effect on plasma cortisol levels in broiler chickens from 8 to 36 d of age *(Olanrewaju et al., 2008)*. *Kalandarov et al. (1984)* reported increased urine levels of adrenaline and 17-oxycorticosteroids in men exposed to 7 ppm of ammonia for 17 days. *Von Borell et al. (2007)* found an increase in plasma cortisol in nursery pigs subjected to acute (96 h) or prolonged (19 days) exposure to ammonia at concentration of up to 50 ppm. Conversely, *Gustin et al. (1994)* reported that pigs exposed to 4 concentrations of ammonia (0.25, 50 and 100 ppm) showed no stress response. The reported stress-inducing effect of ammonia at low concentrations (up to 50 ppm) and the lack of adrenal response to ammonia at concentration of 100 ppm suggest that the effect of ammonia on adrenal function was probably related to its concentration and continuance of exposure. The observed effects of ammonia on adrenal glands activity are most likely to be mediated by nitric oxide (NO). In many investigations it was found that ammonia could induce increased formation of NO in liver and brain *(Kozenko et al., 1995, 1998; Swamy et al., 2005; Chaundhury et al., 2012)*. Different NO donors inhibited basal and adrenocorticotropin-stimulated corticosterone production *(Ducsay et al., 2011).* Also, NO exerted powerful inhibitory effect on steroidogenesis in adrenal glands *(Dave et al., 1997; Drewett et al., 2002; Adams et al., 1992).* In contrast *Mohn et al. (2005)* found that NO increased corticosterone release from adrenals. Immobilization stress was reported to increase neuronal nitric oxide synthase (n NOS) mRNA in the adrenal cortex of rats *(Cymering et al., 2002). Dycsay and Myers (2011)* suggested that adrenal cortical cells express endothelial NOS and that NOS produced locally may play a role in local regulation of cortisol synthesis. The mechanisms underlying ammonia-induced effects on adrenal function are no yet fully understood. Stress response of rabbits to dog barking caused only slight elevation of cortisol in both groups (Figure 1) that was more pronounced in the rabbits, reared under high ammonia level ($P>0.05$). It appears that rabbits may have learned to ignore dog barking since the rabbit farm was guarded by two watch dogs which were kept out of the building near the front door. Alternatively, the observed slight increase of cortisol after exposure of the rabbits to the sound of a barking dog could be related to the reported stressor dependent effect of NO on the response of hypothalamo-pituitary-adrenal axis to exercise and restraint *(Jankort et al., 2009*). Besides, cortisol is demonstrated to stimulate muscle glutamine output *(Barnes et al., 1974*; *Dormann et al., 1988; Hundal et al., 1992).* Urea and glutamine are the two major pathways for ammonia detoxication (*Damink et al., 2002; Hassinger, 1988*). Glutamine on its turn stimulates production of inducible NOS by macrophages *(Li and Moore, 2007).* These data suggest that ammonia implication in the metabolic process may be more complicated than it appear on the surface.

Heterophil to lymphocyte (H:L) ratio in both groups of rabbits was similar (Figure 4) despite the lower basal cortisol level in the rabbits, reared under high ammonia levels.

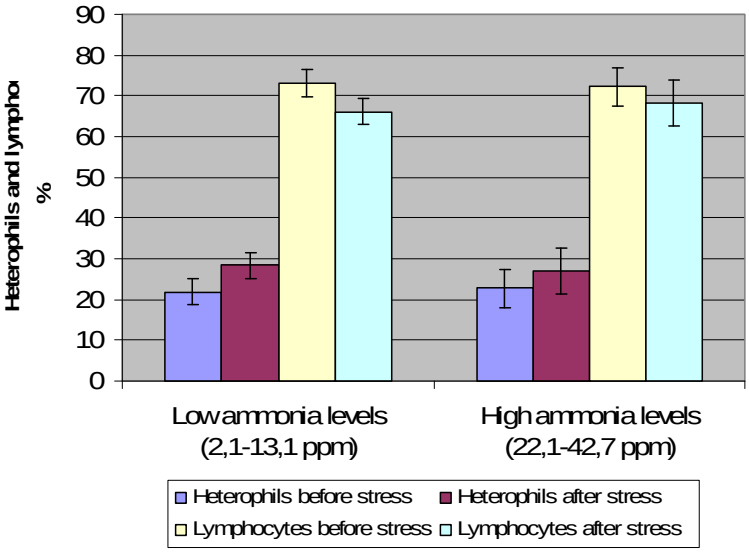

Fig. 4: Mean percentage of heterophils and lymphocytes before and following exposure to stress in rabbits under under low and high indoor ammonia levels (spring)

These results are not consistent with the well documented effect of glucocorticoids on H:L ratio *(Dhabhar e al., 1995).* In our previous study (in press) we suggested that glucocorticoid-induced increase of H:L ratio is probably mediated by the inhibitory effect of glucocorticoids on NO production. However, ammonia unlike glucocorticoids exerts stimulatory effect on NO production and therefore it could compromise the inhibitory effect of glucocorticoids on NO production which would ultimately prevent the effect of glucocorticoids on H:L ratio. There was a trend toward lower H:L ratio in the rabbits under high ammonia levels (0.08) as compared to that in the rabbits under low aerial ammonia (0.13) following exposure to stress (Figure 4). These results suggest that ammonia may have influenced H:L ratio either via its modulatory effect on cortisol synthesis or by its stimulatory effect on NO production. Our view is further supported by the reported increase of the absolute lymphocyte numbers against the background of increased plasma cortisol in weaned pigs exposed to ammonia at concentration of 35 and 50 ppm *(Von Borell et al., 2007).*

Hematocrit value was significantly higher in winter than in summer (Figure 2).

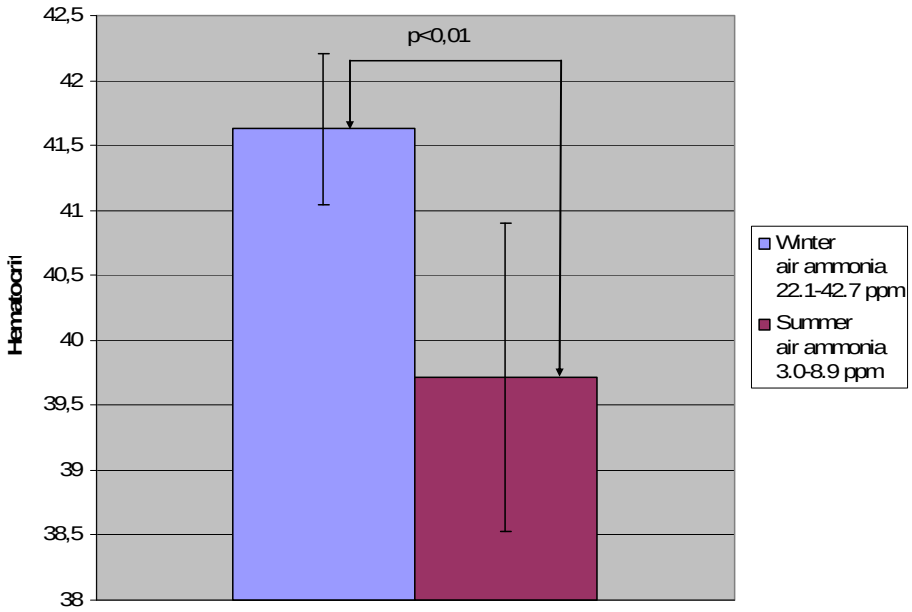

Fig. 2: Seasonal values of hematocrit in rabbits

The impact of season on hematocrit level was most likely due to the effect of temperature. *Chiericato et al. (1994)* reported higher levels of hematocrit in rabbits kept under low temperature (12^0 C) as compared to that in rabbits under high temperature (30^0 C). Also, they found higher level of blood urea nitrogen, in the rabbits kept at low temperature. These metabolic changes were attributed to increased protein catabolism. It could be assumed, therefore that the increased hematocrit level in our study during winter season was influenced by both endogenous and aerial concentrations of NH3. Our suggestion is consistent with the finding of *Olanrevaju et al. (2008)*, who reported increased partial pressure of CO2, hematocrit and hemoglobin on d 6 in broiler chickens exposed to 3 levels (0.25 and 50 ppm) of ammonia concentration for 14 days. The observed enhancement of hematocrit caused by exposure to ammonia was attributed to an impaired oxygen-carrying capacity in the blood leading to compensatory reaction to the lack of oxygen in the tissue. *Sriram et al. (2011)* demonstrated that relatively small changes in hematocrit may significantly raise NO concentration in the vascular wall, thereby causing vasodilation. Exposure of the rabbits to stress had no effect on hematocrit level (Figure 3).

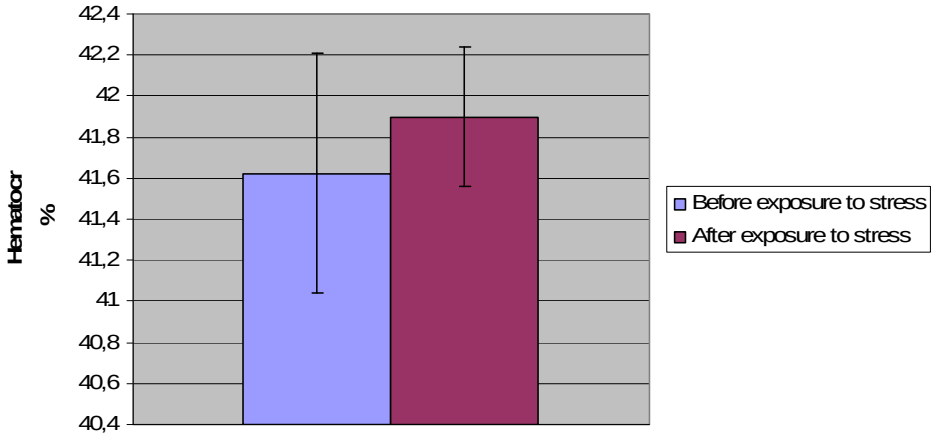

Fig. 3: Hematocrit values in exposed to stress rabbits kept under high ammonia levels (February)

The unchanged hematocrit level after exposure to stress could be attributed to the fact that at the time of the stressor event hematocrit had already been elevated by the prolonged impact of the elevated concentrations of both exogenous and endogenous ammonia in winter months as evidenced by the higher hematocrit in winter relative to that in summer (Figure 2). Prolonged exposure to high aerial ammonia did not effect red blood cell count both in summer and winter season (Table 1) despite the higher hematocrit level in winter (Figure 2). Exposure to stress did not influence red blood cell count.

Table 1. Total leukocyte and erythrocyte count following exposure to stress in rabbits, reared under low and high ammonia levels

Hematological indices	n	Low ammonia levels		High ammonia levels	
		Before stress	After stress	Before stress	After stress
Erythrocyte no (x 10^6/mm^3) in summer	6	6.06±0.046	5.988±0.06	5.941±0.046	5.980±0.058
Leukocyte no (x 10^3/mm^3) in summer	6	6400±144.91	6325±191.7	**a 6507±113.39	6475±147.48
Erythrocyte no (x 10^6/mm^3) in winter	6	-	-	5.993±0.0.54	6.23±0.416
Leukocyte no (x 10^3/mm^3) in winter	6	-	-	**b 6241±131.97	6270±156.52

*Data are presented as means ± standard deviation values with different letters differ (** $p<0.01$)*

Leukocyte numbers in summer were significantly higher than in winter and were probably related with the level of ammonia in winter. The results of this study are not consistent with the reported increase in white blood cell count (WBC) in pigs exposed to ammonia at concentration of 0.35 and 50 ppm *(Von Borell et al., 2007)*. The observed discrepancy could be due to the fact that the reported increase of WBC was accompanied with elevation of plasma cortisol level while in our study basal plasma cortisol declined in winter, when aerial ammonia levels were high. Various noxious stimuli revealed significant changes in leucocyte numbers *(Tronson et al., 1997)* which indicate a possible mediatory role for cortisol. Likewise, our data concerning the effect of ammonia on leukocyte numbers are not consistent with the results of another study, where exposure of pigs to aerial ammonia (0.25, 50 and 150 ppm) did not alter either white blood cell count or plasma cortisol level *(Gustin et al., 1994)*. These data suggest that the modulatory influence of ammonia on WBC was probably mediated by its effect on cortisol secretion.

Summer values of rectal temperature in rabbits reared under high ammonia levels were higher ($P<0.01$) than those in the rabbits reared under low ammonia concentrations (Figure 5). Our results are consistent with the reported higher values of rectal temperature in broiler chickens reared under various aerial concentrations of ammonia (16, 28, 39, 54 ppm). The chickens reared under the highest concentration of ammonia had the highest rectal temperature *(Yahav, 2004)*.

Rectal temperature in the experimental rabbits was higher in summer ($P<0.01$) than in winter (Figure 5) despite the higher concentrations of ammonia in winter relative to summer.

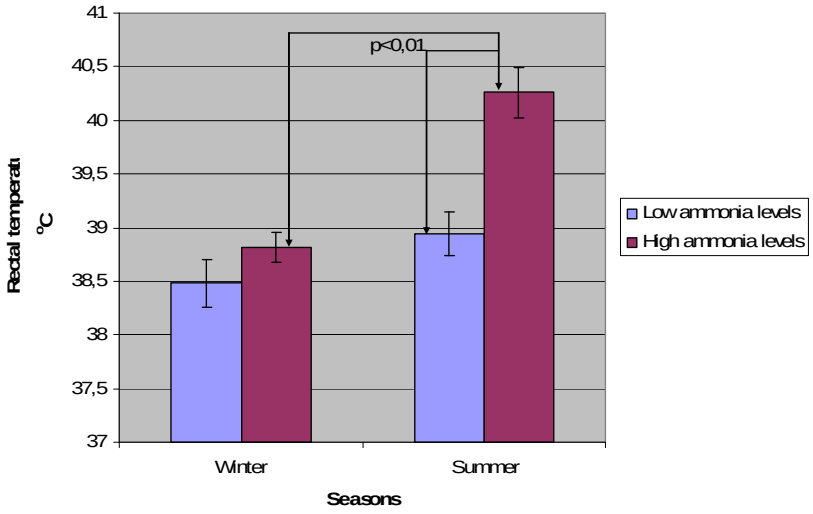

Fig. 5: Effect of season and air ammonia level on rectal temperature in rabbits

This discrepancy could be explained with the stimulatory effect of ammonia on NO production which was reported to influence heat dissipation *(Mills et al., 1997).* It is logical to expect, that rabbit thermoregulation mechanism and thermoregulatory behavior should counteract heat-dissipating effect of ammonia in winter, when air temperature in the room, where the rabbits were housed, was about 4^0 C and there was a need to reduce heat loss in order to maintain thermal homeostasis. It is obvious that thermoregulatory mechanisms were influenced, although in different manner, by aerial ammonia. This view is supported by our previous study (in press) involving rabbits kept at 9-12^0 C. In that study we found significantly lower rectal temperature in the rabbits, reared under high ammonia (22 ppm), relative to that in the rabbits under low aerial ammonia concentration (7 ppm).

Conclusion

Prolonged exposure of rabbits to ammonia resulted in decline of basal plasma cortisol level in spring and increase of rectal temperature in summer. The rabbits had higher hematocrit value in winter and higher leukocyte count in summer.

Acknowledgment

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Funkcionalna aktivnost nadbubrežne žlezde, rektalna temperatura i neki hematološki indeksi kod zečeva odgjenih u zatvorenim objektima sa niskim i visokim nivooima amonijaka

M. Dyavolova, D. Gudev, I. Yanchev, P. Moneva

Cilj ovog eksperimenta bio je da se ispita uticaj amonijaka na nadbubrežnu reakciju, odgovor na stres, odnos heterofila i limfocita, hematokrit, broj eritrocita, broj leukocita i rektalnu temperature kod zečeva. Dvanaest novozelandskih belih kunića, muškog pola (prosečne starosti 4 meseca, a prosečne težina 3 kg) podeljeni u dve grupe, uzgajani su u žičanim kavezima. Zečevi u prvoj grupi, za razliku od onih u drugoj grupi, su odgajeni na nižem nivou gustine naseljenosti. Eksperimenti su sprovedeni u periodu zima-proleće (februar - mart - april) i leto (jul). Nivoi amonijaka u vazduhu u objektu sa većom gustinom naseljenosti bili su 22,1 - 42,7 ppm u sezoni zima-proleće, i 2,1 - 13,1 ppm u leto, dok nivo amonijaka u objektu sa niskom gustinom naseljenosti su znatno niži (2,1 - 13,1 ppm) u sezoni zima-proleće, a u letnjoj sezoni (0.9 - 1.2 ppm). Zečevi su bili izloženi audio stresu 1 min (lajanje pas) u trostrukom 110 dB reprodukciji. Pas koji laje ne prouzrokuje značajno podizanje nivoa kortizola u plazmi. Izlaganje visokom nivoa amonijaka rezultiralo je u padu bazalnog ($p < 0,01$) i stres-indukovanog ($P > 0,05$) nivoa kortizola u poređenju sa odgovarajućim vrednostima kortizola kod zečeva odgajenih pod niskim nivoom amonijak u vazduhu (prolećni eksperiment). Stres je rezultirao u blagom povećanju odnos heterofil i limfocita koji je bio izraženiji kod zečeva odgajenih pod nižim nivoima amonijaka ($P > 0,05$). Zečevi odgajeni pod visokim nivoima amonijaka imaju višu rektalnu temperaturu u letnjem periodu ($p < 0,01$) i zime ($P > 0,05$). Hematokrit vrednosti bile su veće zimi nego leti ($p < 0,01$). Ukupni broj leukocita i eritrocita nisu pod uticajem sezone i nivoa amonijaka. Ova studija pokazuje da dugoročno izlaganje koncentraciji amonijaka inhibira funkciju nadbubrežne žlezde i utiče na termoregulaciju u kunića.

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THE COMPARISON OF NUTRITIONAL AND MICROBIOLOGICAL QUALITY OF CARP (*CYPRINUS CARPIO L.*) AND RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) FARMED IN SLOVAKIA AND POLAND

J. Tkaczewska[1], W. Migdał[1], M. Walczycka[1], P. Kulawik[1], P. Bajzík[2]

[1]Department of Animal Product Technology, Faculty of Food Technology, University of Agriculture in Krakow, ul. Balicka 122, 31-149 Kraków, Poland
[2]Department of Hygiene and Food Safety Faculty of Biotechnology and Food Sciences, Slovak University of Agriculture Tr. A. Hlinku 2 949 76 Nitra Slovakia
Corresponding author: Tkaczewska@gmail.com
Original scientific paper

Abstract: The subject of the study was the flesh of carps and rainbow trouts bred at Slovakian and Polish farms. The influence of location and type of farming on the chemical composition, fatty acid profile and microbiological quality of fish, has been analyzed. It was expected that carp and trout differ in quality depending on location and type of farming. All chemical analyses on study material were performed according to standard methods. The study has shown that basic chemical composition of trout does not depend on the place of breeding and cultivation methods. Fatty acid profiles of carp and rainbow trout differ in quality depending on location and type of farming. It also has been found that Polish and Slovakian carps and trouts have high microbiological quality and high nutrition value.

Key words: carp, trout, nutritional value, place of breeding.

Introduction

Carp is a fish species farmed in Poland for hundreds of years and at present Poland is one of the largest carp producer in the world. The production of rainbow trout is also of considerable sizes although the tradition of trout farming is no longer then forty years *(Ligaszewski, 2006)*.

Despite the fact that Slovakia is situated mostly in the mountain area, which is not an area promoting the fish farming, the total aquaculture production in this country is estimated at around 2 500 tones yearly. Carp and rainbow trout are leading farmed species in Slovakian aquaculture *(European Commision, 2005)*.

The nutritional quality of fish meat depends mostly on the content of protein, mineral components, fat and fatty acid profile. The key elements which for nutritional and technological properties of fish meat are environmental factors in which those animals live *(Puchała and Pilarczyk, 2007)*. Fish farmed in the Slovakia region are from some time on available also on Polish market.

Therefore the purpose of this study was to determine the quality, expressed as the proximate composition, fatty acid profile and microbiological contamination, of the most important farmed fish species in Poland and Slovakia.

Material and Methods

Fish was bought directly from the fish breeders, from the batch designated for the market. From each farm 10 individual carps (*Cyprinus carpio L.*) and 10 individual rainbow trouts (*Oncorhynchus mykiss*) were brought for analysis. The weight of fish varied depending on the place of farming and was 1500 – 2000 g for carp and 330 – 870 g for trout. The farms from which the fish were obtained are located in Poland and Slovakia and are subject to veterinary supervision.

Polish farm: The farm is located in the southern area of the Małopolskie region in the Cracow district. Fish ponds were powered by water from Rudawa river, the left-bank tributary of the Vistula river. Trout were fed with granulated feed mixtures acquired from "Aller-Aqua" company and of the following composition: fish meal, fish oil, hydrolyzed protein, wheat, rapeseed, soy and blood meal. Carps were fed with corn, wheat and barley.

Slovakian farm: The farms were located in the north-west part of the country. The fish ponds of the trout farms were powered in water by the Revúca river, and the carp ponds were powered by the Kamenný Potok river, which is a left-bank tributary of the Turca river. Trout were fed with granulated feed mixtures from the " Le Gouessant" company with the following composition: fish meal, organic soya meal, fish oil, pea, wheat. Carps were fed exclusively with commercial granulated feed mixtures with the protein content, from the fish meal, of 17%.

The analysis of proximate composition (protein, fat, moisture and ash content) was performed according to AOAC (*2000*) methods.

Fatty acid composition was analyzed using the gas chromatography, using the gas chromatograph TRACE GC ULTRA equipped in SUPELCOWAX 10 column. The separation conditions: carrier gas was helium with the flow rate of 5 ml/min, spit flow 10 ml/min, the dispenser temperature was 220^0C, column temperature 200^0C, and detector temperature was 250^0C.

Samples for microbiological analysis were collected according to Polish Standard, PM-ISO 18593:2005. Swabs from the 6.25 cm^2 surface of fish trunk and

transported in tubes with AMIES carbon surface acquired from BioCorp (BioCorp, Warsaw, Poland). The initial suspension and further decimal dilutions were prepared according to Polsih Standards PN-ISO 18593:2005 and PN-EN ISO 6887-3:2005. Prepared dilutions were used to perform the microbiological analysis. The analyses consisted of: aerobic plate count (APC) (according to PN-EN ISO 4833:2004/Ap1:2005), coagulase positive *Staphylococcus* count (according to PN-EN ISO 6888-1:2001/A1:2004), *Pseudomonas spp.* count (PN-ISO 13720:1999) and *Escherichia coli* count (PN-ISO 16649-2:2004).

Statistical analysis of the results was performed by using the statistical package Statistica 10.0 (StatSoft, Tulsa, Oklahoma, USA). The normality of the results was tested using Saphiro-Wilk test. The significance of difference was established by t-Student test and nonparametric U Mann-Whitney test. All analyses were performed in duplicate repetitions (10n x 2).

Results and Discussion

Fish meat quality is determined by a number of factors, among which the content of fat, protein and ash is very important. Moisture analysis of carps tissue acquired from different farms showed highly significant difference ($p<0.01$) depending on the place of cultivation, while the amount of moisture present in trout meat was not dependent on the place of cultivation (Table 1).

Table 1.Proximate composition of trout and carp from farms in Poland and Slovakia

Trout		
Content	**Poland X average±SD**	**Slovakia X average±SD**
Water [%]	76.28^{a}±298	77.27^{a}±0.72
Total Protein [%]	18.82^{a}±0.87	18.87^{a}±1.04
Fat [%]	2.67^{a}±0.25	2.88^{a}±0.23
Ash [%]	1.46^{a}±0.25	1.26^{b}±0.05
Carp		
	Poland	**Slovakia**
Water [%]	78.24^{a} ±1.75	83.27^{b}±1.45
Total Protein [%]	16.90^{a}±1.01	14.96^{b}±1.28
Fat [%]	3.77^{a}±0.33	0.56^{b}±0.02
Ash [%]	1.07^{a}±0.28	0.95^{b}±0.01

$^{A, B, C}$ – significance of difference $p<0.05$
$^{a, b, c}$ – significance of difference $p<0.01$

The meat of Slovakian carp has higher moisture content (83.27%) then meat of the carp cultivated in Poland (78.24%). According to *Friedrich and Stepankowska (2001)* the moisture content in cultivated carp is at the level of 69-72%. *Grela et al.* (*2010*) studied carps meat rom Lubelskie region in Poland, harvested at different time (from September till October) and determined the moisture content of carp meat was 77.79 – 78.86%. *Filipiak et al. (1998)* determined the moisture content of carp meat was from 61.0-69.9%. According to those authors, the high difference of moisture content in carp tissue is dependent on the fat content in the feed for fish.

The moisture content in the rainbow trout meat from different farms ranged from 76.26-77.27%. Other studies show the moisture content of rainbow trout is from 72 - 73% *(Mhongole 2009, Wogu and Maduakor 2010)*. However *Çelik et al. (2008)* report much lower moisture content (65 - 71%). This study did not show any significant correlation between the moisture content of meat and a type of feed. Other conclusions were drawn by *Sealey et al. (2011)*. They analyzed the proximate composition of trout meat fed with significantly different feeds (the protein and fat content originate from different sources). The research showed that the fat and moisture content was dependent on the type of used feed.

Fish products are one of the best sources of animal proteins *(Sikorski, 2004*). The analysis of protein content in meat of carps meat originated from different farms showed its highly significant differences ($p<0.01$), depending on the type and area of cultivation, while the protein content in trout meat was not affected by the place of cultivation. Meat of carp from Polish farms contained 16.90% of proteins compared to 14.96% in carps cultivated in Slovakia. The results are similar to the results obtained in other studies *(Grela et al., 2010; Hossain et al., 2001)*. *Białowąs (2007a)* states that there is a strict correlation between the protein content in fish feed and the protein content in fish meat. Carps cultivated in Polish farms were fed with cereal feeds, while carps cultivated in Slovakia were fed with commercially prepared feed mixtures.

Also the protein content in rainbow trouts meat cultivated in both regions is similar to the results available in the literature. Different researchers *(Çelik 2008, Skibniewska and Zakrzewski 2008, Usydus et al., 2011)* founf out the amount of protein was 16 - 19%. *Sealey et al. (2011)* indicates that protein content in trout meat is dependent of its amount in feed, while the source of protein (fish meal or natural feed) has no influence the protein content in the fish tissue. Those studies showed the protein content in trout meat from 21.70 – 23.20% which is higher than the results obtained in our study.

Lipid content in carp meat showed highly significant difference depending on the region of cultivation ($p<0.01$). Meat of carps from Polish farm contained 3.77% fat, while meat of carps from Slovakian farms only 0.56%. The difference in

fat is influenced probably by changes in moisture content of analyzed fish *(Puchała and Pilarczyk, 2007)*. *Czerwińska (2007)* and *Friedrich and Stepankowska (2001)* show the fat content of carp meat from 4.2 – 17% while other authors state that fat content of carp is from 0.5-3.0% *(Skibiniewska and Zakrzewski 2008; Grela et al., 2010)*. Such diverse results in studies prove that the fat content of carp is an individual feature and strictly depends on the cultivation conditions.

The fat content in the meat of rainbow trout did not vary depending on the farm from which they were acquired. According to other research *(Türker and Yildirim 2011; Usydus 2011)* the fat content of rainbow trout is 5.5-8.8% which is different than the results obtained in this study. It can be related to the season of catch. This study was performed during spring, when fish have higher energy expenditures related to incensed preying *(Sikorski 2004)*.

The ash content in the meat of rainbow trout and carp from different farms was highly significantly different ($p<0.01$). The carcasses of carps from Polish region showed 1.07% of ash, while carps from Slovakina region only 0.95%. *Hossain et al. (2001*) showed more than double amount of ash in carp tissue (2.6%). The study performed by *Grela et al.* (*2010*) however shows the ash content of 1.07-1.25%. Also trout cultivated in Poland show significantly higher content of ash (1.46%) compared to the trout cultivated in Slovakia (1.26%). Other studies show similar results in ash content of trout meat. *Çelik at al.* (*2008*) and *Yildiz et al.* (*2004*) show the ash content 1.20 – 1.60%. *Borucka-Jastrzębska et al.* (*2009*) state that the place of cultivation of freshwater fish (both carp and rainbow trout), significantly affects the mineral composition of their tissue (Mg, Ca, Zn, Co, Fe). The acquired results confirm the conclusions made by *Borucka-Jastrzębska et al. (2009*).

The benefits from fish consumption result mostly from the adequate fatty acids composition in their meat, particularly from the high content of n-3 fatty acids (mostly eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) (*Usydus et al. 2011*).

This study shows highly significant ($p<0.01$) influence of the area of cultivation on the fatty acids composition in the meat of carp and rainbow trout (Table 2).

Table 2. Fatty acid profile of trout and carp from farms in Poland and Slovakia

Trout		
	Trout Poland	Trout Slovakia
SFA	23.95[a]	17.55[b]
UFA	71.94[a]	80.35[b]
UFA/SFA	3.01[a]	4.58[b]
MUFA	37.57[a]	41.07[b]
PUFA	34.37[a]	39.28[b]
PUFA/SFA	1.44[a]	2.24[b]
PUFA/MUFA	0.92[A]	0.96[A]
PUFAn3	24.90[a]	16.72[b]
PUFAn6	8.59[a]	22.15[b]
n3/n6	0.34[a]	1.32[b]
OFA	20.40[a]	14.66[b]
DFA	74.61[a]	82.71[b]
PI[1]	128.88[a]	178.94[b]
AI[2]	0.26[A]	0.27[A]
TI[2]	0.28[A]	0.24[B]
Carp		
	Carp Poland	Carp Slovakia
SFA	21.74[A]	23.40[B]
UFA	77.83[a]	75.25[b]
UFA/SFA	3.58[A]	3.23[B]
MUFA	45.71[a]	30.35[b]
PUFA	32.12[a]	44.90[b]
PUFA/SFA	1.48[a]	1.93[b]
PUFA/MUFA	0.70[a]	1.48[b]
PUFAn3	13.28[a]	21.36[b]
PUFAn6	18.32[a]	22.72[b]
n3/n6	1.37[a]	1.06[b]
OFA	17.39[A]	17.03[A]
DFA	81.28[A]	79.99[A]
PI[1]	105.01[a]	173.90[b]
AI[2]	0.44[a]	0.26[b]
TI[2]	0.23[A]	0.19[B]

[A, B, C] – significance of difference with $p<0.05$

[a, b, c] – significance of difference with $p<0.01$

OFA – hypercholesterlemic fatty acids (C14:0 + C16:0)

DFA – neutral and hypocholesterolemic fatty acids (C18:0 + UFA)

[1] *PI*= (% monoenic acids x 0.025) + (% dienic acids x 1) + (% trienic acids x 2) + (% tetraenic acids x 4) + (% pentaenic acids x 6) + (% heksaenic acids x 8) *Ulbricht and Southgate (1991)*

[2] AI=(C12:0 +(4xC14:0) + C16:0) / [(MUFA + ∑SPUFA (n-6) + (n-3)]

[2] TI = (C14:0 + C16:0 + C18:0) / [(0.5 x MUFA + 0.5 x SPUFA (n-6) + 3 x SPUFA (n-3) + (n-3)/(n-6)] *Arakawa and Sagai (1986)*

Highly significant difference ($p<0.01$) between the content of saturated (SFA), unsaturated (UFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids in the meat of carps and trout acquired from different farms was recorded.

WHO recommends that fat should constitute of around 30% of daily energy intake and only 10% should constitute from SFA (*WHO, 1999*). The highest content of SFA was found in carps from Slovakia (23.07%). The amount of UFA was inversely related to the amount of SFA in meat. The highest amount of MUFA was found in the meat of carps from Polish farm (45.71%) while the highest amount of PUFA was in carps cultivated in Slovakia (44.90%). Other studies show different ratio of each fatty acids group in carp meat. *Jaeen and others (2011)* states hat carp meat contains 55.6% of SFA, 32.9% of MUFA and only 11.42% of PUFA. *Afkhami et al. (2011*) and *Grela et al. (2010*) show results similar to the results obtained in this study: 21 - 35% of SFA, 21.18 - 48.57% of MUFA and 21.46-23.00% of PUFA.

Trout meat from acquired from Polish farm was more rich in SFA (23.95%) and contained less MUFA (37.57%) and PUFA (34.37%) compared to trout meat from Slovakian aquaculture. The place of cultivation significantly affects the lipid composition of rainbow trout meat. Similar conclusions have been drawn by *Erdem et al. (2009*) who studies the fatty acids composition of seabass (*Dicentrarchus labrax L.*) cultivated in different regions of Turkey. They reported significant differences in the content of SFA, MUFA and PUFA in tissue of fish acquired from different cultivation area. Also *Çelik et al. (2008*) during their study concluded that the microclimate conditions in which the fish were cultivated significantly affects the fatty acids composition of fish meat.

Highly significant differences ($p<0.01$) were found in the n-6/n-3 fatty acids ratio in the meat of carps and rainbow trout obtained from farms in Poland and Slovakia.

The AI, TI and PI obtained in this study are significantly lower than the results obtained in other research showing fatty acids profile of different farmed animals. *Dal Bosco et al.,* (*2004*) reported that AI and TI of rabbit meat are 0.7 and 0.99 respectively. Also the poultry and pigeons meat show higher AI and TI then the meat of fish in this study *(Dal Bosco, 2005)*. *Dal Bosco et al.* (*2012*) analyzed the meat of goldfish (*Carassius auratus)* and acquired the PI of 185-228. AI of 0.35-0.44 and TI of 0.22-0.30 which is similar to the results obtained in this study.

Despite significant differences in fatty acid composition of carps and trout acquired from different farms, it can be concluded that the fat of fish present in the meat of fish from all studied farms is characterized by high nutritional value.

There are two main threats related to the presence of microorganisms in food of aquatic origin. First threat is caused by saprophytic organisms, which when

present in higher numbers can cause the deterioration of sensory features and the total deterioration of the product. The second threat is related to the presence of pathogens, which can cause food poisoning, harmful for the welfare of the consumers *(Kołżyn-Krajewska, 2007)*.

Amount and type of microorganisms present on the surface of the fish body is the reflection of microbiological quality of water, in which the fish live (*Bett 2010*). The primary microflora of the aquatic food products are the organisms causing deterioration. Microbiological spoilage of fish meat is faster than the spoilage of meat from other warm-blooded animals stored in the same conditions.

The APC of the microflora present on the body surface of carps and rainbow trout was not significantly different in fish from different farms (Fig 1)

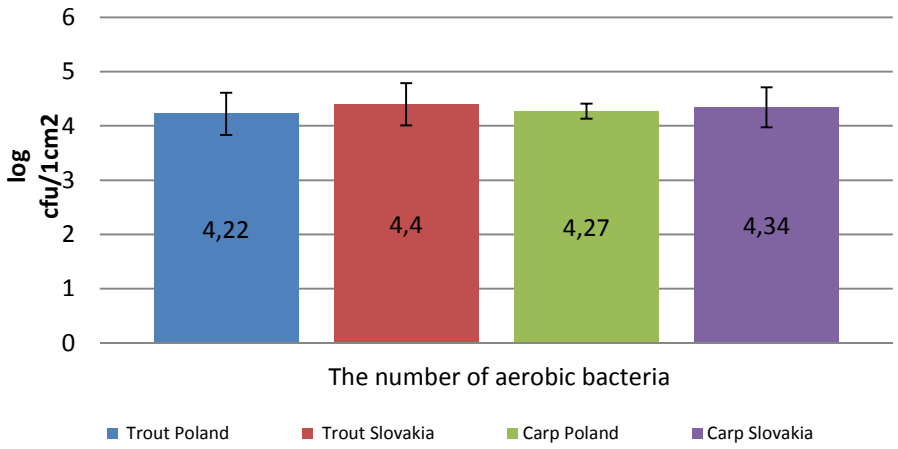

Figure 1. The APC on skin of carp and trout from farms in Poland and Slovakia

Wogu and Manduakor (2010) studied the amount of microorganism in carcasses of different aquacultural fish species. They reported the amount of microorganisms on fish skin at the level of 7.04-7.33 log cfu/1cm^2. The research found also that the amount of microorganisms on skin is much higher than in meat or gills. Lower counts of microflora on the fish skin was obtained by *Mhongole (2009*), who reports the APC for those fish as 2.38-6 log cfu/1cm^2 and by *Adams and Moss (2008)*, who found 2-7 log cfu/1cm^2 of fish skin. Other research show similar results to the ones obtained in this study. *McAdams et al. (1996)* studied the APC of microrganisms on the surface of rainbow trouts cultivated in different waters (recirculated water and slow flow). They reported the APC of 3-6 log cfu/1cm^2 of fish skin. The 10^5 cfu/1cm^2 is regarded as the amount of microflora that is still satisfactory in terms of hygiene *(McAdams, 1996)* .

Table 3. The amount of *Staphylococcus aureus. Pseudomonas spp. Escherichia coli* on skin of carp and trout from farms in Poland and Slovakia

	Trout Poland	Trout Slovakia	Carp Poland	Carp Slovakia
Stphylococcus aureus	The number of bacteria lower than 10 cfu per 1cm^2 on skin surface of fish			
Pseudomonas spp.				
Escherichia coli				

The amount of coagulase positive *Staphylococcus* on the whole surface of carps and rainbow trout from studied farms were lower than 10 cfu/1cm^2 (all the incubated colonies scored negative on coagulase test), which indicates o threat to the consumers (Table 3). *Wogu and Maduakor (2010)* isolated the strains of *Staphylococcus aureus* from the body surface of fish cultivated in aquaculture, *Zmysłowska et al.* (*2002*) did not find any coagulase positive *Staphylococcus* on the surface of the carp, but they reported the presence of this bacteria in the digestive tract of carp (1.46 log cfu/g).

The bacteria from *Pseudomonas sp*. strains commonly occur in the nature. They are part of indigenous microflora of fresh water. *Pseudomonas spp.* count on the surface of carps and trouts was lower than 10 cfu/1cm^2. Since some strains of the *Pseudomonas* are psychrotrophic with protelytic properties, this bacterium can affect the quality and shelf life of fish during cold storage. During storage the bacteria penetrate through damaged skin into the meat tissue and their metabolic activity quickly leads to spoilage *(Mhongole, 2009)*.

Escherichia coli count in all analysed samples was lower than 10 cfu/1cm^2. *Escherichia coli* are not present on the surface of fish just after catch. The exception is only in fish acquired from farms, where water is highly contaminated with animal or human excrements *(Mhongole, 2009)*. According to *Harnisz and Tucholski (2010) Escherichia coli* can be present in the fish mucus and in their digestive tract in waters with low contamination. In such cases however the presence of this bacterium is not noted in inner parts of meat and organs.

The results acquired from microbiological analysis of carp ad rainbow trout skin indicate good hygienic state of water in all studied farms.

Conclusions

- The meat of rainbow trout acquired from aquacultures located in the regions of Poland and Slovakia shows similarities in proximate composition

- The proximate composition of carp meat varies depending on the area of cultivation
- Fatty acids composition of carp and trout meat varies depending on the place of cultivation
- Carp and trout acquired from studied aquacultures do not pose a microbiological threat for the consumers, since no pathogenic bacteria were found on the skin of the animals.

Poređenje nutritivnog i mikrobiološkog kvaliteta šarana (*Ciprinus carpio l.*) i pastrmke (*Oncorhinchus mikiss*) uzgajanih u Slovačkoj i Poljskoj

J. Tkaczewska, W. Migdał, M. Walczycka, P. Kulawik, P. Bajzík

Rezime

Predmet ispitivanja je bilo meso šarana i pastrmke koji se uzgajaju u ribnjacima u Slovačkoj i Poljskoj. Uticaj lokacije i vrste proizvodnje na hemijski sastav, profil masnih kiselina i mikrobiološki kvaliteta ribe je analiziran u ovom radu. Očekivalo se da će se šaran i pastrmka razlikovati po kvalitetu u zavisnosti od lokacije i vrste proizvodnje. Sve hemijske analize su izvršene u skladu sa standardnim metodama. Studija je pokazala da osnovni hemijski sastav pastrmke ne zavisi od mesta uzgoja i gajenja. Profili masnih kiselina šarana i pastrmke se razlikuju u kvalitetu u zavisnosti od lokacije i vrste proizvodnje. Takođe je utvrđeno da poljski i slovački šaran i pastrmka imaju visok mikrobiološki kvalitet i visoku hranjivu vrednost.

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RESEARCH ON INCIDENCE AND EFFECTS OF VIRAL ARTERITIS ON HEALTH STATUS OF HORSES AT STABLES

S. Lazić[1], D. Lupulović[1], G. Lazić[1], P. Gavrilović[2], D. Bugarski[1], T. Petrović[1]

[1] Scientific Veterinary Institute „Novi Sad“, Rumenački put 20, 21000 Novi Sad, Republika Srbija
[2] ***Veterinary Specialists Institute*** „Pančevo“, Novoseljanski put 33, 26000 Pančevo, Republika Srbija
Corresponding author: lazic@niv.ns.ac.rs
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Abstract: This article presents the results of an investigation of equine viral arteritis conducted at six horse stables in the territory of Vojvodina. The research encompassed analysis of clinical manifestation of the disease, seroprevalence and virus detection in native semen samples. Analysis of clinical manifestations of the disease was performed according to the anamnestic data obtained from staff veterinarians on the farms. Seroprevalence of equine viral arteritis was determined by examining 150 blood serum samples applying the serum neutralization method. Seropositive finding was obtained on one horse farm. Out of the total of 51 analysed blood serum samples, positive result was confirmed in 17 horses. The established antibody titre values ranged from 2 to 10 $\log_2$. Native semen samples were obtained by collecting three subsequent ejaculates from two stallions from this stables, one with seropositive and one with seronegative status. The samples were collected at 24-hour intervals and analysed using RT-PCR technique. Presence of the virus was confirmed in all three native semen samples originating from seropositive stallion.

Key words: equine viral arteritis virus, horse stables, seroprevalence, semen

Introduction

Equine viral arteritis (*Equine viral arteritis*–EVA) is an infectious disease that can affect all horse categories. The disease occurs worldwide causing severe health problems and considerable economic losses. The causal agent of EAV is *Equine arteritis virus*–EAV from the family *Arteriviridae*. EAV is an enveloped, spherical ***virus*** of 50-70 nanometres (nm) in diameter possessing single-stranded ribonucleic acid (RNA) genome of 12.7 kb *(Balasuriya et al., 1999)*. The virus was

first isolated in 1953 in *Bucyrus*, *Ohio* state, U.S.A. *(Nowotny, 2001)*. The name of the virus, arteritis, is based on the characteristic pathogenic activity observed in blood vessels. The virus replicates in muscle cells and endothelial cells of small blood vessels, predominantly arteries, and may lead to the range of inflammatory processes.

According to data from the literature *(Holyoak et al., 2008)*, the disease has been reported in horse populations in all continents: North and South America, Europe, Asia, Australia, Africa. Only Iceland and Japan are considered EVA-free. A research that dates back to the 60ies and 70ies of the last century has established the following seroprevalence rates of EAV: Switzerland 11.3%, England 2.3%, Netherlands 14% and Germany 1.8%. However, the investigation dated 1998 revealed seroprevalence rates of some 20% in Germany and about 2% in non-vaccinated horses in the U.S.A. *(Holyoak et al., 2008)*. In Austria, seroprevalence of EAV ranges between 10 and 12%; however, on some horse stables the rate reaches even 100% *(Nowotny, 2001)*. Seroprevalence rate in Anatolia (central Turkey) is 23.4% *(Bulut et al., 2012)*. According to the available literature, there are no data on seroprevalence of EAV in the territory of the Republic of Serbia. In our country, the research on EAV has been conducted only since the beginning of this century encompassing only imported horses put in quarantine. More recently, the disease has been gaining more attention and numerous horse breeders have put efforts to initiate serological testing, foremost of breeding animals.

Infected horses shed the virus in nasal and ocular discharge, urine, aborted foetuses and sperm (stallions). The virus can cause a range of health disorders in horses, including interstitial pneumonia, panvasculitis with consequent oedema (predominantly in eye lids, abdomen, distal parts of the limbs, preputium and scrotum), thrombosis associated with bleeding, necrosis in lymph nodes and kidneys, abortions, inflammation of accessory sex glands. Sometimes, the infection may pass without developing any health status disorders *(Del Piero, 2000)*. Infected stallions are potential natural reservoir of the virus. If the virus affects accessory sex glands, stallions remain infected for life and frequently shed the virus in the semen *(Del Piero, 2000; Nowotny, 2001; Holyoak al., 2008)*. Investigation on virus spreading in the semen and its detection in the semen was the topic of interest of numerous researches. Development of modern laboratory diagnostic methods, such as biomolecular assays, gave rise to more intense research in this field, which have confirmed that seropositive stallions shed the EAV in their semen *(Ramina et al., 1999; Balasuriya et al., 2002; Mankoč et al., 2002; Guthrie et al., 2003; Chenchev et al., 2010)*.

Material and methods

Samples

Blood samples were collected from six horse stables (designated with letters A-F) from the territory of Vojvodina Province in the period fall/winter 2012/2013. Samples were collected by venipuncture of the *Vena jugularis* in to Vacutainer tubes, and serum separation was performed after spontaneous coagulation. Anamnestic data on health status during the past 3-year period were recorded for each horse subjected to blood sampling. Thus, a total of 150 blood samples and anamnestic records on investigated horses were obtained.

Semen samples were collected from two stallions from the horse stables B, where artificial insemination of mares was practiced. Native semen ejaculate used for artificial insemination is collected by using an artificial vagina (*Missouri* model, Germany). Native semen sampling was performed after completed serological examination, i.e. in April 2013. Semen samples were collected from one EAV-seropositive and one seronegative stallion. A 3-mL sample for virology testing was obtained from three subsequent ejaculates that were collected from both stallions at 24-hour intervals using an artificial vagina.

Analysis

Identification of EAV-antibodies in blood serum samples was performed by the method of virus neutralization, according to procedure set out by *OIE Terrestrial Manual, Chapter 2.5.10, 2008*. Virus neutralization was performed by using ***continuous*** rabbit-kidney ***cell line*** RK-13 and *Bucyrus* strain of EAV. According to the OIE Terrestrial Manual, antibody titre values ≥ 2 $\log_2$ are considered seropositive result. Animals that do not have a certified history of vaccination against EVA are considered infected, i.e. such stallions are considered potential source of infection.

Detection of EAV RNA: RNA was extracted from each of 6 homogenized/lysed native stallion semen samples using a commercial kit (Qiagen Viral RNA Mini Kit, QIAGEN, Germany) according to manufacturer's instruction. Native semen, artificially contaminated with the Bucyrus reference strain of EAV (100 $TCID_{50}$/0.1 mL), was used as a positive control for RNA extraction and RT-PCR. The obtained RNA extracts were further amplified by reverse transcription – polymerase chain reaction (RT-PCR) using primers 5'-CAGCGAATTTAAAC GCTTTTGAAC-3´ and 5'-AGTAGTTCGCCTGTGTGAGC-3' that target virus nucleocapsid gene described by *Balasuriya et al*. (2002). One-step RT-PCR amplification was done using commercial kit Qiagen OneStep RT-PCR kit chemistry (QIAGEN, Germany) in 25 µl reactions according to manufacturer's

instruction. Specific PCR product of 204 bp was detected and visualised by electrophoresis on 1.5 agarose gel with ethidium bromide.

Results and discussion

To provide a clear overview of our findings, the obtained results are presented in Tables.

Table 1. Overview of examined horses according to the stables and EVA antibody finding

No.	Horse farm designation	No. of horses on the farm	No. of examined horses on the farm	Result	
				Positive	Negative
1	A	120	20	0	20
2	B	62	51	17 (33%)	34 (66%)
3	C	72	25	0	25
4	D	20	17	0	17
5	E	33	16	0	16
6	F	68	21	0	21
Total		375	150	17 (11%)	133 (89%)

According to data displayed in Table 1, the examination encompassed 150 horses (40%) out of the total of 375 horses from six horse stables in Vojvodina. Seropositive finding was obtained on one horse stables (B), where 51 out of 62 horses were examined, and seropositive finding was confirmed in 17 (33%) of examined animals.

Equine arteritis virus infection has been established only on stables B. The analysis of the results obtained on this farm can illustrate the effects of this infection on the health status of horses, incidence of the infection within different horse categories as well as the potential for virus dissemination on the horse farm.

According to anamnestic data obtained from staff veterinarians and farm manager, during the past 3-year period clinical symptoms indicating equine viral arteritis were not observed. Abortions were recorded only sporadically and were mainly associated with twin-embryo pregnancies. Frequently, mares from other farms were introduced to the farm for breeding, mostly lacking the documents of serological testing for sexually transmitted infectious agents.

Incidence of infection and the established EVA antibody titre values within different horse categories were displayed in Table 2.

Table 2. Antibody titre values within different categories of examined horses at the stables B

Horse category	No. of examined horses	No. of horses with established antibody titre (log_2) from:										
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
Stallions	9	5	0	2	0	0	0	0	1	0	1	0
Mares	20	7	1	0	0	3	4	3	1	0	0	1
Castrates	4	4	0	0	0	0	0	0	0	0	0	0
3-year-olds	8	8	0	0	0	0	0	0	0	0	0	0
2-year-olds	10	9	0	0	1	0	0	0	0	0	0	0
Total	**51**	**33**	**1**	**2**	**1**	**3**	**4**	**3**	**2**	**0**	**1**	**1**

Table 3. Antibody findings at stables B according to stallions' age

Stallion age	No. of examined stallions	No. of horses with established antibody titre (log_2) from:										
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
under 4 y	1	1	0	0	0	0	0	0	0	0	0	0
5-7 y	3	2	0	1	0	0	0	0	0	0	0	0
8-10 y	2	2	0	0	0	0	0	0	0	0	0	0
11 y and above	3	0	0	1	0	0	0	0	1	0	1	0
Total	**9**	**5**	**0**	**2**	**0**	**0**	**0**	**0**	**1**	**0**	**1**	**0**

Table 4. Antibody findings at stables B according to mares' age

Mare age	No. of examined mares	No. of horses with established antibody titre (log_2) from:										
		0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
under 4 y	4	4	0	0	0	0	0	0	0	0	0	0
5-7 y	2	2	0	0	0	0	0	0	0	0	0	0
8-10 y	7	0	0	0	0	4	1	1	1	0	0	0
11 y and above	7	1	1	0	0	0	2	2	0	0	0	1
Total	**20**	**7**	**1**	**0**	**0**	**4**	**3**	**3**	**1**	**0**	**0**	**1**

According to the results presented in Table 2, antibodies against EAV were established in stallions and mares and in only one 2-year-old animal. Analysis of the established antibody titre values in stallions and mares according to their age category indicates that stallions older than 10 years, which are the breeding ground of this stables (i.e. most frequently used for breeding), are infected with EAV. Among the infected stallions on stables B, seropositive finding was established in only one stallion aged 6 years. Hence, four out of the total of nine stallions from the stables B were infected with EAV. Within the mare population, EAV infection is predominant in animals older than 8 years. In these animals, high antibody titre

values were established, with a mean titre value being 5.42 $\log_2$. However, analysis of breeding records (artificial insemination during 2012) reveals that these mares were inseminated with the semen originating from two seropositive stallions. High EAV antibody titres (7 and 9 $\log_2$) were established in both stallions. Analysis of the health status of both stallions and mares in 2012 did not reveal any disorders, early-term or late-term abortions. The foaling occurred at expected term in all mares, and newborn foals did not manifest any health status impairments.

RT-PCR assay confirmed presence of EAV in all samples of native semen (3) of a seropositive stallion, whereas (using the same examination method) EAV could not be detected in samples obtained from seronegative stallion. Antibody titre of stallion with EAV-positive ejaculate was 9 $\log_2$.

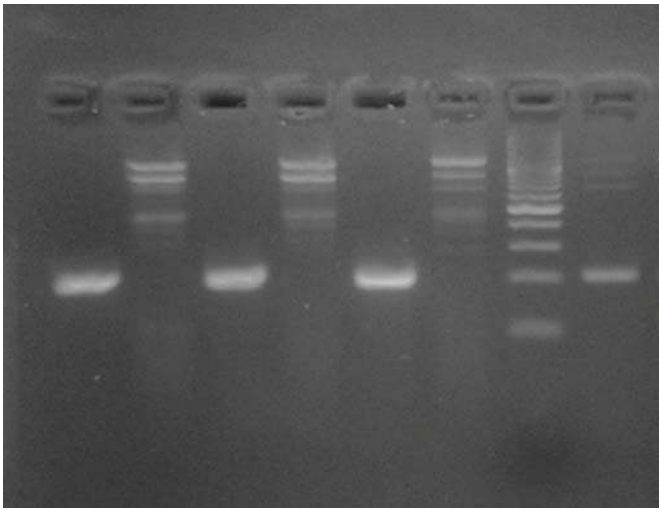

Figure 1. RT-PCR products from semen samples (from the left to right):1. Semen of seropositive stallion collected 24 Apr., 2. Semen of seronegative stallion collected 24 Apr., 3 Semen of seropositive stallion collected 25 Apr., 4. Semen of seronegative stallion collected 25 Apr., 5. Semen of seropositive stallion collected 26 Apr., 6. Semen of seronegative stallion collected 26 Apr., 7. Marker 100 bp and 8. EAV, strain Bucyrus, as a positive control

Analysis of the results obtained in this research revealed that they well correspond with the data from the literature. High seroprevalence on some horse stables reported by *Nowotny (2001)* and several other researchers is due to an unobstructed virus transmission within the farm. Virus shedding in the semen of seropositive stallions is frequently reported by numerous authors *(Mankoč et al.,*

2002, Chenchev et al., 2010), thus high rate of seroconversion in mares inseminated with semen originating from seropositive stallions was expected result.

Conclusions

Out of the six horse stables with total 375 horses encompassed by this research, EAV infection was established at one stables. The seroprevalence of equine viral arteritis was 27.42%. In three native semen samples collected from one seropositive stallion (three subsequent ejaculates collected at 24-hour intervals), the presence of EAV was confirmed in a RT-PCR assay.

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Ispitivanje raširenosti i uticaja virusnog arteritisa na zdravstveno stanje konja u ergelama

S. Lazić, D. Lupulović, G. Lazić, P. Gavrilović, D. Bugarski, T. Petrović

Rezime

U radu su prikazani rezultati ispitivanja bolesti virusnog arteritisa konja u 6 ergela na teritoriji Vojvodine. Ispitivanja su vršena sa aspekta kliničke manifestacije bolesti, seroprevalence i utvrđivanja virusa u uzorcima nativne sperme.

Analiza kliničke manifestacije bolesti vršena je na osnovu anamnestičkih podataka dobijenih od veterinarskog osoblja ergela. Utvrđivanje seroprevalence virusnog arteritisa konja vršeno je ispitivanjem 150 uzoraka krvnih seruma, metodom serum neutralizacije. Seropozitivan nalaz utvrđen je na jednoj ergeli. Od pregledanih 51 uzoraka krvnih seruma, pozitivan nalaz utvrđen je kod 17 konja. Utvrđena vrednost titra antitela iznosila je od 2 do 10 $\log_2$. Sa ove ergle kod dva pastuva, sa seropozitivnim i seronegativnim nalazom, u uzorcima nativne sperme iz tri uzastopna ejakulata, uzorkovana u vremenskim intervalima od 24 sata, RT-PCR tehnikom, dokazan je virus u sva tri ejakulata seropozitivnog pastuva.

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Institute for Animal Husbandry
Autoput 16, P. Box. 23, 11080, Belgrade-Zemun, Serbia
Tel: +381 11 2691 611; +381 11 2670 121; +381 11 2670 541;
Fax: + 381 11 2670 164;
biotechnology.izs@gmail.com
www.istocar.bg.ac.rs