UDC636

Print ISSN 1450-9156
Online ISSN 2217-7140

BIOTECHNOLOGY IN ANIMAL HUSBANDRY

CONTENTS

VOL 29, 1
Founder and publisher
INSTITUTE FOR ANIMAL HUSBANDRY
11080 Belgrade-Zemun
Belgrade 2013

Journal for the Improvement of Animal Husbandry

UDC636

Print ISSN 1450-9156
Online ISSN 2217-7140

BIOTECHNOLOGY IN ANIMAL HUSBANDRY

Belgrade - Zemun 2013

Biotechnology in Animal Husbandry 29 (1), p 1-181, 2013 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 636

Address of the Editor's office
Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080 Belgrade-Zemun, Republic of Serbia
Tel. 381 11 2691 611, 2670 121; Fax 381 11 2670 164; e-mail: biotechnology.izs@gmail.com; www.istocar.bg.ac.rs

Biotechnology in Animal Husbandry is covered by Agricultural Information Services (AGRIS) -Bibliographic coverage of abstracts; Electronic Journal Access Project by Colorado Altiance Research Libraries -Colorado, Denver; USA; Matica Srpska Library -Referal Center; National Library of Serbia; University Library "Svetozar Markovic", Belgrade, Serbia; EBSCO, USA; DOAJ and European Libraries

According to CEON bibliometrical analysis citation in SCI index 212, in ISI 9, impact factor (2 and 5) of journal in 2012: 0,667 and 0,467, - M24 category

Annual subscription: for individuals -500 RSD, for organizations 1200 RSD, -foreign subscriptions 20 EUR. Bank account Institut za stočarstvo, Beograd-Zemun 105-1073-11 Aik banka Niš Filijala Beograd.

Journal is published in four issues annually, circulation 100 copies.

The publication of this journal is sponsored by the Ministry of Education and Science of the Republic of Serbia.
Printed: "Mladost birošped", Novi Beograd, St. Bulevar AVNOJ-a 12, tel. 381 11 2601-506

Biotechnology in Animal Husbandry 29 (1), p 1-17 , 2013 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 636
DOI: 10.2298/BAH1301001P

POTENTIALS OF SERBIAN LIVESTOCK PRODUCTION - OUTLOOK AND FUTURE

M.M. Petrović[1], S. Aleksić[1], M. P.Petrović[1], M. Petrović[2], V. Pantelić[1], Ž. Novaković[1], D. Ružić-Muslić[1]

[1]Institute for Animal Husbandry, Belgrade – Zemun, 11080 Zemun, Serbia
[2]Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Zemun, Serbia
Corresponding author: Milan M.Petrović, e – mail: milpet99@gmail.com
Review paper

Abstract: The paper describes the state of the livestock production in the Republic of Serbia including preliminary results from the 2012 census: according to preliminary data, 908.990 heads of cattle, 3.403.288 pigs, 1.729.278 sheep and 235.576 goats are reared in Serbia. Structural and institutional measures, and measures of credit support should be the main instruments for achieving the goals of progress in animal husbandry. Activities in the breeding-selection work should be carried out in accordance with the recommendations of international organizations (ICAR, INTERBULL, EAAP). One of the most important outcome of these activities should result in a level of over 80% of the total population of cattle, pigs, sheep and other species of domestic farm animals in Serbia included in the control of production performance (presently between 25 and 30%). Genetic improvement of cattle, sheep and pigs (milk, meat) is very complex. More efficient use of reproductive technologies (AI - artificial insemination and embryo transfer ET, etc.), also of methods for evaluation of breeding value of farm animals through new methods of evaluation of breeding value of cows and breeding bulls will contribute to faster genetic improvement of production traits of these species of domestic animals. New knowledge in mapping and gene transfer, marker assisted selection, *in vitro* embryo development, embryo cloning, sexing, etc., are improving rapidly, with new technologies being developed permanently. Breeding/improvement of cattle, sheep and pigs in through breeding – selection work should facilitate further improvement of fertility traits, growth rate, feed efficiency, carcass quality (higher percentage of muscle tissue in the body), the quality of milk and meat, resistance to disease and stress, etc..

Key words: cattle, sheep, pigs, breeding, genetics, ET, MOET

Introduction

In early 2013, preliminary results were obtained in the agricultural census in the Republic of Serbia, carried out in 2012. According to the conducted census, there are 631.122 farms in Serbia as follows: 2.567 holdings of legal entities and entrepreneurs and 628.555 family farms/agricultural holdings. Total area of utilized agricultural area is 3.355.859 ha. The total number of cattle 908.990, 3.403.288 pigs, sheep and goats 1.729.278 and 235.576 heads, respectively. Based on these data and the data for 2011. (sbs, 2012) differences can be observed. The differences were greatest in the number of sheep, according to the 2012 census, there is nearly 300.000 more heads of sheep. Family households make up 99.6% of total households.

Changes in the global livestock production are very dynamic. In developed countries it is stagnant while demand for animal food products has increased in developing countries causing the increase of livestock production. Rising demand in developing countries is a great opportunity for livestock production. Increased demand in these countries is caused by population growth, urbanization and higher incomes of the population of these countries (*Delgado, 2005*). In the future, production will be limited by natural resources, particularly land and water. Improvements in breeding, nutrition and health of domestic animals will contribute to an increase of the genetic potential in production and improved efficiency of livestock production.

The share of livestock production in total agricultural production and the number of livestock units per hectare of agricultural land, indicate the degree of a country's agricultural development (*Petrović 2005., Petrović et al., 2011.*). Serbian agriculture farming is indispensable. It involves significant natural and human resources. Therefore, the intensification of livestock production and increase of participation in agricultural production are the basic requirements and the need to overcome the present backwardness, provide food to satisfy the demand of the population, stop the imports and make thorough preparations in the provision of quality meat produced for domestic and export purposes (*Aleksić et al., 2007*). The Republic of Serbia has significant natural resources (agricultural land, air, water, etc.) and very significant capacity and resources (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. Livestock production in the future should be able to respond to conditions similar to those in developed countries. The Serbian agriculture is dominated by small farms with an average area between 3 and 4 ha. Process of increasing the efficiency of livestock production may also contribute to the linking of stage of production and processing in a single production cycle,

which requires forming of the association of producers. 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). Serbia has about 0.60 ha of agricultural land and 0.50 ha of arable land per capita, which is above the average in Europe

Production of milk and meat, number of cattle

Cattle breeding absorbs most of the plant products as those of high quality, but also less valuable by-products, turning them into high value products, thus enabling the use of those areas that could not be used without the cattle. This type of farm animal gives the highest production of milk and meat, the basic high protein foodstuff used in human consumption and as raw material in food industries. About 60% of animal protein in human food is provided, in developed countries, through the use of bovine products, ie. milk, meat and meat products. Two breeds of cattle make the basis of the cattle industry in Serbia: Simmental cattle or domestic spotted in Simmental type, which is most common in rural areas on family farms. This widespread breed of combined traits corresponds to the nutritional resources, as well as the current economic situation of producers and the market situation of milk and meat. The weakness in the future, most likely will be lack of specialized breeds in areas where adequate production potential exists for animal feed (fattening/meat breeds - the mountainous region of Serbia). Black and Red Holstein-Friesian breed (dairy type cattle), is mainly present in the organized manufacturing farm production which supplies raw milk to the dairy industry. Numbers of cattle in recent decades, and especially in the last decase has been showing the trend of drastic decrease. This should not be too much alarming in regard to milk production, because the implementation and use of new biotechnological methods, techniques and technologies of breeding (genetic improvement) and increasing milk yield per cow, as well as with the organization of commodity farmers, in the existing cattle population, the production per cow and total production will intensify. However, the drastic decline in the number of females has negative effect on meat production (fewer cows – less calves or less fattening cattle intended for meat production).

Table 1. Numbers of cattle by Category (000 heads)

Year	1985	1990	1995	2000	2005	2010	2011
Cattle TOTAL	1.483	1.246	1.106	1.087	1.057	938	937
Cows	847	759	622	602	578	498	459
Fattening cattle	168	118	223	187	216	185	184

Source: Statistical Office of the Republic of Serbia

Milk production was not accompanied by pronounced downward trend in the number of cattle. The reason is the continued increase in production per cow. Current production is about 1.400 million liters of milk from which a portion of the food consumed by household members and producers sell at green markets, and most of the milk produced is delivered to dairy industry. People in Serbia have very low per capita milk consumption (about 200 liters). Residents of EU countries have far higher per capita consumption of 950 in Denmark and 280 liters in Bulgaria

Table 2. Production of milk in Serbia

Year	Bovine milk (million litres)	Sheep milk (million litres)
1990.	1.805	20
2000.	1.585	19
2005.	1.602	16
2010.	1.462	9
2011.	1.434	11

Source: Statistical Office of the Republic of Serbia

The total average annual production of beef for the past twenty years in Serbia was about 110,000t, with a tendency of decline, especially in recent years, and the latest data show that it is about 80,000 tons. Exports of cattle for slaughter and beef dropped drastically to meet less than 20% of its quota to the EU (8700 tonnes). Production of this type of meat in the Republic of Serbia has varied from 156,000 in year 1985 to 81,000 t in 2011. This quantity is sufficient meat for the domestic market, as the average consumption per capita is about 12 kg. Lower production of beef compared to Serbia have some Scandinavian countries, Portugal, Bulgaria, Greece, etc..

Table 3. Production of meat in Serbia (in 000 tons)

Year	Beef	Mutton	Pork	Total produced
1985.	156			
1990.	139	23	282	444
2000.	104	19	283	406
2010.	92	23	269	384
2011.	81	24	271	376

Source: Statistical Office of the Republic of Serbia

Cattle breed structure

Changes in the breed structure in cattle population raised in Serbia over the past decade has been intense. More intensive breeds have suppressed the extensive breeds and breeds with poor production, so that they had numerically reduced. Now Simmental and Domestic Spotted in Simmental type make up to about 75% of total population, the group of Black-and-White and Red-and-White Holstein-Friesian cattle around 20%, while the primitive races and crosses make up about 5% of the total number of cattle in the Republic of Serbia. According to estimates, of the total 450.000 cows, there are aaround 330.000 heifers and cows of Simmental breed in Serbia, Black and White and Red around 90.000 and around 30.000 of others.

Production of milk and meat, number of sheep

Sheep production is mainly in the possession of small family farms and in herds of 10-15 animals, from 70-100 heads, and fewer farms have 200-500 heads (*Petrović et al., 2011*).

Table 4. Number of sheep (000 head)

Year	1990	2000	2005	2010	2011
Sheep TOTAL	2.127	1.611	1.556	1.475	1.460

Source: Statistical Office of the Republic of Serbia

The average production of sheep milk (Table 2) steadily declined from 20 million liters in 1990 up to 11 million liters in 2011. The total average annual production of sheep and lamb meat for the last 20 years is constant and amounts to about 22.000 t (Table 3). This data shows a very low level of consumption of meat in Serbia of about 3 kg per capita per year.

Sheep breed structure

In terms of breed structure various strains of Pramenka make up about half of the total number of sheep, various types of crosses - about 25%, Tsigai breed about 5% and about 20% imported pure breeds used as improvement breeds. On the territory of the Republic of Serbia, the following sheep strains are reared: Sjenica, Svrljig, Pirot, etc., Tsigai breed sheep, various more or less well-established crosses of different strains of Pramenka sheep and Merino breed. Of improvement sheep breeds in Serbia Merinolandshaf sheep flocks are reared (Wurttemberg breed) as a general improver breed of domestic pramenka strains, Ile de France, Bergamo and

Suffolk, as domestic sheep breed improvers to increase the yield and quality of meat.

Production of pig meat, number of pigs

Number of pigs changed from year to year. In the twenty-year period, the largest number of pigs was reared in 1990 - 4.301 million (Table 5) and the lowest in 2011 - 3.489 million pigs. These data show that for 20 years the number of animals was reduced by one million.

Table 5. Number of pigs (000 head)

Year	1990	1995	2000	2005	2010	2011
Pigs TOTAL	4.301	4.170	4.066	3.870	3.631	3.489
Sows and breeding gilts	708	-	-	-	623	616

Source: Statistical Office of the Republic of Serbia

The main product is high-quality pork. Besides a number of important characteristics of pigs, one of them, which is different from other domestic animals is a considerable amount of meat that can be produced per sow per year (more than 2000 kg of live weight of fatteners or over 1600 kg of carcass sides or more than 800 kg of meat). The importance pig breeding in the world, in Europe and in our country is the fact that of the total production of meat, pork accounts for 40 - 50%. Table 3 shows that the annual production of pork in the twenty-year period (1990-2011) in average was 282. 000 (1990) to 271.000 tons (2011).

Pig breed structure

On farms in Serbia, meat pig breeds and crossbreds are reared. In our swine herds, Landrace breeds (Swedish, Dutch, German, Belgian, Danish), Large White/Yorkshire, Duroc and Pietrain are reared. The most numerous are the breeds Swedish Landrace and Large White/Yorkshire. The share of meat breeds used as the terminal breeds in crossing (Belgian Landrace, German Landrace, Pietrain, Hampshire, Duroc) is low (less than 1% per individual breed). In addition to pure breeds, crosses are produced which make up more than 60% of the total number of sows (*Petrović, 2006*).

HOW TO IMPROVE THE LIVESTOCK PRODUCTION IN SERBIA

Improvement of livestock production should be set as a number of basic directions and clear goals. The following should be implemented: (change the structure of producers, property and institutions, market development and market

mechanisms, rural development and environmental protection) in order to achieve the basic goals that will result in:

- The formation of a sustainable and efficient livestock production that can compete in other markets, contributing to the growth of national income.
- Provision of food of animal origin that meets the needs of consumers in terms of quality and safety.
- Provide support for sustainable rural development.
- Preserve the environment from the effects of livestock production.
- Preparation of livestock production for Serbia's EU integration.
- Preparing policy and domestic support for trade in livestock and WTO rules (*Petrović, 2005*).

Economic policy

For faster and more economically efficient livestock production (milk, meat, etc.), as well as faster and more efficient organizations and associations of farmers, basically it is necessary to set up and solve the problems that have affected negatively, and still are, destabilizing the overall livestock production, motivation of farmers, the purchasing power of consumers and the regular supply of the market. Increasing the competitiveness of Serbian livestock production can be achieved by creating the conditions for the market environment through investment, both in knowledge and in equipment. Therefore, structural and institutional measures, and measures of credit support should be the main instruments for achieving this goal. Market measures, in an indirect way, may also contribute to achieving this goal (export subsidies, direct support prices, direct payments per hectare / per head of cattle and subsidizing inputs indirectly, by reducing the cost of agricultural products). Structural measures (investment support program through grant a certain percentage should support investment in equipment and machinery to improve production (milking equipment and storage of animal products, facilities, and equipment for raising cattle, sheep and pigs). Lending support through short-term, and especially long-term loans should result in the realization of this goal, as they allow investment in livestock, equipment and new technology.

Selection activities in livestock production

In the future, the activities of the breeding-selection work should be carried out in accordance with the recommendations of international organizations dealing with origin and production traits in farm animals (ICAR, INTERBULL, EAAP and other). In accordance with the EU regulations, a series of steps should be made to

produce formation of: a single database for all types of farm animals, and the system model for monitoring of production data, modeling of the evaluation of breeding values - unique breeding programs, support the organization of associations of breeders of farm animals with the creation of quality requirements/preconditions for organized work through the activities of associations through alliances at the national level and according the species of farm animals, to improve work of performance and progeny centers for testing and production of bull semen, boar semen, etc.

One of the most important results of this activity should result in a level of over 80% of the total population of cattle, pigs, sheep and other species of domestic animals in Serbia included in the control of production performance and traits (now between 25 and 30%).

Genetic improvement of cattle

The main reasons for the increase in domestic production of milk and meat are: the current production is insufficient to meet domestic demand, including potential exports. In Serbia, the number of cattle and cows must be increased (at least 600.000 head of cattle), the breeding-selection work in cattle production must be modernized. Genetic improvement of cattle (milk, meat) is very complex. Firstly, by increasing herd size per farm, conditions for greater genetic progress for more efficient production must be provided. More efficient use of reproductive technologies (AI - artificial insemination and embryo transfer – ET, etc.), and the use of statistical methods for estimating breeding values of farm animals through new methods of evaluation of breeding values of bulls and cows, new traits, which are subject to selection and greater intensity and effect of selection (*Simm et al., 2004*) will contribute to faster genetic improvement of milk production of cows. Linear methods are the basis for determining the breeding value of candidates for selection. The best linear unbiased indicators BLUP with a single and multiple random genetic effects can be divided into individual model, AM, a sire model, reduced animal model - RAM, and for more variables, models with the same and different descriptive matrices, with and without missing data and models with different data on relatives. The latest multi variable model -(test day model, which among other things uses curve and persistency of lactation.

Determining the variances and covariances of the basic population is done using a REML model (restricted maximum likelihood). In addition to milk traits that fall into the primary, the selection should be extended to other secondary traits. To body development characteristics and type, which for a long time have been included in the selection, health, traits of fertility and longevity, etc., are added (*Vollema, 1998*). The incidence of mastitis is a real problem in intensive and high yielding milk production, and among other things it can be reduced through

selection (somatic cells), inspite of the low heritability (0.05 to 0.10). For this purpose, the number of daughters per bull in progeny testing is increased. According to *Vollema (1998),* heritability of longevity is low (0.10) with the provison that it increases later in life, but it is considered reasonable to pay attention to in the selection, particularly since the correlations between certain properties of body development and longevity are positive. New knowledge in mapping and gene transfer, selection markers through in vitro embryo development, embryo cloning, sexing, etc., very rapidly increasing, with an effort to develop new technologies (*Bulfield, 1998*).

Status and cattle breeding objectives in Serbia. Due to the reduction in the number of cattle and the production of milk and meat per animal and in total, essential programs and resources for reconstruction and development of cattle breeding are necessary. Instead of traditional small producers with two or three cows, which are rapidly extinguished, as many modern commodity farmers as possible should be developed (*Petrović and Lazarević, 2003*). Milk production should be developed in intensive conditions and meat production in a variety of conditions, from extensive to intensive. The breeding programs for improvement of Black and White and Simmental cattle need to be updated and successfully realized with the use of the massive introduction of modern biotechnology in reproduction (AI, ET, MOET). Our cattle populations should be kept open in terms of the breeding but to develop own bull test and selection work and connect with the participation and cooperation with international organizations and programs. In addition to our selection within populations, a controlled introduction of quality genetic material and crossing should be performed in order to achieve faster genetic improvement (*Petrović et al., 2006*). The breeding objectives are: Simental breed, the average milk production in standard lactation over 6.000 kg with 4.10% milk fat and 3.60% protein; Holstein Friesian breed in standard lactation over 8.000 kg with 4.00% fat and 3,50% protein (*Pantelić et al., 2010*). A possible way to improve fattening and slaughter traits in domestic Simmental breed is the systematic crossbreeding with beef cattle breeds, using the effect of diversity breeds, heterosis effect (*Simm, 1998*) - crosses obtained from crossing domestic cattle of lower production capacity and bulls of beef breeds, French (Charolais, Limousine, Blonde d'Aquitaine), Italian (Chianina, Piemontese), English (Aberdeen Angus, Galloway, Hereford). The breeding objectives of this work and the use of heterosis effect is the creation of such genotypes that will allow final body weight of F1crosses - young cattle from over 550 kg, average daily gain in the fattening of over 1500 g, warm carcass yield of over 60%, the content of the muscles in the body of over 65% (*Petrović et al., 2007; Petrović et al., 2011*).

Status and sheep breeding objectives in Serbia. Development of sheep production requires a large number of activities: increasing the number of sheep; forming of nucleus herd for certain breeds of sheep, as a way of creating of main population; implementation of flock selection and protection of native sheep breeds in terms of conservation of genetic resources, biodiversity and national biological heritage. In this respect, we should establish a breed standard (selected heads of sheep); increase ther use of poorly utilized meadows and pastures. These resources, along with measures of improvement, can provide a good basis for the cheaper production of milk and meat. The main limitations in regard to restoration of these resources is the current process of depopulation in mountainous areas.

Breeding sheep to improve meat yield. In order to achieve a certain genetic progress in sheep breeding in Serbia, it is necessary to implement a programmed selection of breeds that are reared. Bearing in mind that the variability of quantitative traits within a population is expressed and that a number of individuals (+ variants) achieve above-average production results, breeders in countries with developed sheep production approached the formation of the nucleus (selective core), which is the backbone of future development and our sheep production.

In order to increase the production of meat, in addition to breeding and selection in pure breed, breeding/crossing methods are implemented in order to utilize the heterosis and create new, more productive breeds of sheep. This implies the following: the crossing of domestic Pramenka sheep with Merinolandschaf rams, breeding crosses that are the optimum combination of these two breeds with each other, crossing two-breed crosses with the third, the terminal breed of fattening type (Ile de France) to improve the fattening traits of lambs, crossing of Tsigai breed with rams of beef breeds (Suffolk, Ile de France and others.) - create a new breed of sheep for meat production *(Petrović et al., 2009.)*

Breeding to increase meat production should enable getting lambs with higher daily gain (over 300 g), higher final body weight at 90 days of age (over 28 kg), as well as greater carcass yields, (more than 58%). In the future period, the work on the problem of sheep reproduction should be intensified, and thus production of lambs for export and domestic markets. Lambing system should be organized so as to provide multiple pregnancy or postpartum period in ewes and productive life and more lambs per birth. The problem of transition to polycyclicity and fertilization of sheep throughout the year would be solved in two ways: genetically, using the crossing of breeds that manifested estrus outside the normal season (Dorzet Horn, Ile De France and Romanovski) using the method of stimulation, ie. induction and synchronization of estrus. In this way, the conditions for the application of intensive lambing system - two lambs in a year or three lambing in two years, would be created. Lambing system, the system of crossing, selection and line breeding, then shortening of the post-partum interval and the

increased lambing and number of lambs at birth, early weaning of lambs, the use of artificial insemination methods, automatic feeding machine, rearing of lambs using successful milk replacer and fattening of lambs from 30 - 45 kg from 90 to 120 days, will form the basis of modern technology in Serbian sheep breeding.

Status and pig breeding objectives in Serbia. The consequences of the future Serbia's entry into the WTO will be an international competition, the prices of pigs and pork products will likely fall more than the price of inputs. The answer to such a state will be a need to reduce production costs (primarily feed for fattening and piglets), which can be achieved by technological improvements, lower variable and fixed costs. In this regard, intensive family farms will have the advantage, but with the assumption that the above technological improvements are fulfiled, but also of economic and agricultural policies. Production standards are related to the fertility of sows and increase the efficiency of feed conversion. This will certainly be influenced by improved production properties and increase in the number of pigs under selection and expansion of artificial insemination of sows. Countries with developed pig breeding produce per sow 22-24 fatteners per year with and tendency of increase and reduction of losses. Researchers predict the production of 30 fattening pigs per sow per year. At 28 days of lactation, gestation period of 114 days and the duration of the period between weaning and 10 days of fertilization, it is possible to achieve a 2.4 farrowing per sow and year. Sows of poorer reproductive performance reduce the average number of parities per sow per year and therefore this parameter is not just a simple sum of the reproduction cycle. In the assumption that the average 10 piglets born alive per litter, losses during lactation 10%, during the breeding and fattening of 5% and 3%, it is possible to produce 19.9 fattening pigs per sow per year in our swine herds (*Petrović et al.,2012*).

Breeding pigs to improve meat yield through breeding-selection work should facilitate further improvement of fertility traits, growth rate, feed efficiency, carcass quality (higher percentage of muscle tissue in the body), and meat quality of pigs resistant to disease and stress. Constant, systematic and planned breeding-selection is necessary to increase the genetic potential of existing meat breeds and crossbreeds of pigs, to create a line of pure breeds in the application of divergent selection, in order to later have the expression of greater heterosis effect in their crossing. The aim of selection should be the increased muscle tissue in the most valuable parts of the carcass: leg, shoulder and loin. The breeding program in our country provides for crossing of Landrace (Swedish, Dutch) and Large White to produce the gilts of F1 generation. They are then crossed with boars of the third race (terminal sire breeds: Hampshire, Duroc, Pietrain, German and Belgian Landrace) or boars of F_1 generation (*Petrović et al., 2006*).

It is necessary to choose the best farms in order to establish elite, breeding (reproduction) and production (commercial) herds in the pyramid organization of

production of breeding pigs and fatteners. This is necessary because it is not clearly defined which farms can produce quality breeding sows and boars, what the ratio should be with regard to the number of heads and the path of movement of pigs from the top to the bottom of the pyramid, in order to achieve greater genetic progress. The selection differential must be maximized and short generation interval. In these herds only breeding in pure breed is applied. Reproduction (breeding) herds of pigs are used to reproduce the pure breed because the elite herds can not produce a sufficient number of breeding animals. They purchase tested and positive evaluated animals from elite herds. Genetic improvement of traits at the top of the pyramid is extended to a larger number of animals. In addition to increasing the number of purebred sows, in these herds crossing and production of gilts F1 generation is applied. In these herd, the production traits of pigs are tested and selection implemented. The criteria are somewhat lower than in the elite herds. Breeding animals from these herds are used for the production of fattening pigs in farms and production by private producers. Production (Commercial) herds produce only fattening pigs. They acquire male and female reproductive breeding animals from the herd. Gilts of F_1 generation can be purchased from reproductive herds, boars can be purchased from the elite herds or boar seed can be purchased of crosses or purebred animals from AI centers. Progeny of the three breed (three line) and / or four breed (four line) crosses are intended only for fattening. Why is the high fertility that is expected from the maternal line important? It is important because the sale must pay the costs of pig production piglets, keeping sows and boars, gilts and the production of pigs. Increasing the number of fattening pigs per sow per year enables to reduce the number of sows on the farm.

Boars from AI centres can be used for breeding in pure breed, producing crossbred sows in reproductive herds and produce commercial hybrids. In this way it is possible to achieve economic gains. Nucleus herds are selling quality breeding animals, the Center for AI greater amount of semen and commercial herds provide quality of animals for fattening and therefore should allocate funds from each of the slaughtered finishing pigs for breeding-selection work. Profit is the reduction of production costs associated with production of fattening pigs, high quality meat products and quality breeding animals are produced for the market.

NUTRITION. Nutrition is the most important paragenetic factor, and it should follow the genetic potential of the animal. In the next period, because of the price of final products of animal origin, special attention should be focused on this area (feed accounts for 50-60% of the cost of products), which ultimately should lead to significant rationalization in Serbian livestock production, i.e. production of milk and meat. Transfer of technological developments in this field in the world and our excellent experience should be placed in the supporting function of improved and new genotypes of domestic animals so that genetic potential of animals could be

manifested. 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. 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 conditions of preserved natural environment, cattle and sheep will in the best possible way 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.). Technological solutions in the field of nutrition of fattening cattle and sheep will depend on many factors, primarily the specific areas (plains, hills and mountains). Requirements of domestic animals in terms of carbohydrates, protein, minerals and vitamins are known. In the future, the results of modern biotechnological methods in the field of nutrition and physiology should be used, so that they contribute to the development of livestock production and to contribute to the profession in general through better understanding of the processes in the field of nutrition and especially high genetic potential of animals and how they relate to reproductive performance (*Butler, 2000, Dumas et al., 2008*). Efficiency of livestock production fuels permanent research in the field of nutrition. Limitations and instability in production for farmers will represent the increase in feed conversion efficiency per kg of gain, which is one of the basic parameters of profitability.

In other words, milk producers will have to find cheaper and more competitive opportunities in nutrition, to reduce production costs, (example: a higher proportion of feed from meadows and pastures and increase the use of corn silage and some by-products of the food industry).

Conclusion

The paper describes the current condition in livestock production of the Republic of Serbia including preliminary results from the 2012 census. The total numbers of various farm animal species are following: cattle 908.990, pigs 3.403.288, sheep 1.729.278 and goats 235.576.Structural and institutional measures, and measures of credit support should be the main instruments for achieving the goals of progress in animal husbandry. Activities in breeding-selection work should be carried out in accordance with the recommendations of international organizations (ICAR, INTERBULL, EAAP). One of the most important results of this study should result in a higher level of over 80% of the total population of cattle, pigs, sheep and other species of farm animals in Serbia included in the control of production performance (now between 25 and 30%). Genetic improvement of cattle, sheep and pigs (milk, meat) is very complex. More

efficient use of reproductive technologies (AI - artificial insemination and embryo transfer ET, etc.), and use of methods for estimating breeding values of domestic animals through new methods of evaluation of breeding value of cows and breeding bulls will contribute to faster genetic improvement of production traits of these species of farm animals. New knowledge in mapping and gene transfer, marker assisted selection, *in vitro* embryo development, embryo cloning, sexing, etc., are improving rapidly, with new technologies being developed permanently. Breeding/improvement of cattle, sheep and pigs through breeding – selection work should facilitate further improvement of fertility traits, growth rate, feed efficiency, carcass quality (higher percentage of muscle tissue in the body), the quality of milk and meat, resistance to disease and stress, etc.

Acknowledgement

Paper financed by the Ministry of Education, Science and Technological Development of Republic of Serbia, Project TR - 31053

Mogućnosti stočarstva Srbije – perspektiva i budućnost

M.M. Petrović, S. Aleksić, M. P. Petrović, M. Petrović, V.Pantelić., Ž. Novaković, D. Ružić-Muslić

Rezime

Početkom 2013. godine dobijeni su preliminarni rezultati popisa poljoprivrede u Republici Srbiji, obavljenog 2012. godine. Ukupan broj goveda je 908.990, svinja 3.403.288, ovaca 1.729.278 i koza 235.576 grla**.** Povećanje konkurentnosti stočarstva Srbije se može ostvariti kreiranjem uslova za tržišno okruženje i putem investicija, kako u znanje, tako i u opremu. Zbog toga strukturne i institucionalne mere, kao i mere kreditne podrške treba da budu glavni instrumenti za ostvarenje ovog cilja. Aktivnosti u odgajivačko-selekcijskom radu treba da se sprovode u skladu sa preporukama međunarodnih organizacija (ICAR, INTERBULL, EAAP i druga). Jedan od najvažnijih rezultata ovog rada treba da prouzrokuje nivo od preko 80% ukupne populacije goveda, svinja, ovaca i ostalih vrsta domaćih životinja u Srbiji bude obuhvaćeno kontrolom proizvodnih svojstava (sada između 25 i 30%).

Status i ciljevi oplemenjivanja goveda u Srbiji. Genetsko unapredjenje goveda (mleko,meso) je vrlo kompleksno. Efikasnije korišćenje reproduktivnih

tehnologija (VO-veštačko osemenjavanje i ET-embriotransfer i dr.) i korišćenje metoda za procenu odgajivačke vrednosti domaćih životinja preko novih metoda ocene priplodnih vrednosti bikova i krava će doprineti bržem genetskom unapređenju mlečnosti krava. Nova saznanja u mapiranju i transferu gena, selekciji preko markera, in vitro razvoju embriona, seksiranju i kloniranju embriona i dr. Odgajivački ciljevi-simentalska rasa, prosečna proizvodnja mleka u standardnoj laktaciji preko 6 000 kg; Holštajn frizijska rasa u standardnoj laktaciji preko 8 000 kg. Melezi iz ukrštanja domaćih krava nižih proizvodnih sposobnosti i bikova tovnih rasa, francuskih, italijanskih, engleskih. Odgajivački ciljevi ovakvog oplemenjivačkog rada i korišćenje heterozis efekta je stvaranje takvih genotipova koji će omogućiti završnu telesnu masu meleza F1, junadi od preko 550 kg, prosečni dnevni prirast u tovu preko 1500 g, randman toplih polutki preko 60%, sadržaj mišića u trupu od preko 65%.

Status i ciljevi oplemenjivanja ovaca u Srbiji. Oplemenjivanje u cilju povećanja proizvodnje mesa treba da omogući dobijanje jagnjadi sa većim dnevnim prirastom (preko 300 g), veće završne mase tela sa 90 dana uzrasta (preko 28 kg), kao i većeg randmana trupa, više od 58%). Problem prelaska na policikličnost i oplodnju ovaca u toku cele godine obavio bi se na dva načina: genetički, korišćenjem ukrštanja rasa koje manifestuju estrus izvan normalne sezone (dorzet horn, il de france i romanovska) primenom metoda stimulacije, tj. indukcijom i sinhronizacijom estrusa.Tako bi se stvorili uslovi za primenu intenzivnog sistema jagnjenja dva jagnjenja u jednoj godini odnosno tri jagnjenja u dve godine. Sistem jagnjenja, sistem ukrštanja, selekcija i linijsko odgajivanje, zatim skraćenje post partum intervala i povećanje indeksa jagnjenja, kao i broja jagnjadi pri rođenju, rano zalučenje jagnjadi, korišćenje metode veštačkog osemenjavanja, automatskih mašina za dojenje, odgajivanje jagnjadi uspešnom zamenom za mleko i tov jagnjedi od 30 - 45 kg sa 90 do 120 dana.

Status i ciljevi oplemenjivanja svinja u Srbiji. Oplemenjivanje svinja u cilju unapređenja mesnatosti preko odgajivačko-selekciojskog rada treba da omogući dalje poboljšanje osobina plodnosti, brzine porasta, iskorišćavanja hrane, kvaliteta trupa (veći procenat mišićnog tkiva u trupu), kvaliteta mesa i otpornosti svinja na bolesti i stres. Odgajivačko-selekcijskim radom neophodno je: povećati genetski potencijal postojećih mesnatih rasa i meleza svinja, raditi na stvaranju linija unutar čistih rasa primenom divergentne selekcije, kako bi se kasnije njihovim ukrštanjem ispoljivo veći heterozis efekat. Cilj selekcije treba da bude povećanje mišićnog tkiva u najvrednijim delovima trupa: but, plećka i kare. Odgajivački program u našoj zemlji predviđa ukrštanje landrasa (švedski, holandski,) i velikog jorkšira radi proizvodnje nazimica F1 generacije. One se posle toga ukrštaju sa nerastovima treće rase (terminalna rasa nerastova: hempšir, durok, pietren, nemački i belgijski landras) ili nerastovima F1 generacije.

References

ALEKSIC S, PETROVIC., M.M., SRETENOVIC LJ., PANTELIC V.,TOMASEVIC D (2007): Cattle production - current situation and future directionin Republic of Serbia. Biotechnology in Animal Husbandry, Book 1, 23, 5-6, 1-11.

BULFIELD G. (1998): Will animal breeding become biotechnology. Proceedings of the 6th World congress on genetics applied to livestock production, vol. 23, p. 19.

BUTLER, W. R. (2000): Nutritional interactions with reproductive performance in dairy cattle. Anim. Reprod. Sci.60–61, 449–457.

DELGADO C. (2005): Rising demand for meat and milk in developing countries: implications for grasslands-based livestock production. In Grassland: a global resource (ed. McGillowayD. A.), p. 29–39. The Netherlands: Wageningen Academic Publishers.

DUMAS , A., DIJKSTRA J., FRANCE J. (2008): Mathematical modelling in animal nutrition: a centenary review. J. Agric. Sci. 146, 123–142.).

PANTELIĆ,V., SAMOLOVAC, Lj., ALEKSIĆ,S., TRIVUNOVIĆ,S., PETROVIĆ,M.M., OSTOJIĆ-ANDRIĆ, D., NOVAKOVIĆ, Ž (2010): Heritability of type traits in first calving Black and White cows, Archiv fur Tierzucht, Vol 53 (2010), 5, 545-554.

PETROVIĆ M.M., BOGDANOVIĆ V., PETROVIĆ P.M., RUŽIĆ-MUSLIĆ D.,OSTOJIĆ D. (2002): Mogućnosti unapređenja stočarstva brdsko-planinskog područja Srbije, Biotechnology in Animal Husbandry, 18 (5-6), p. 1-8.

PETROVIĆ M. M, LAZAREVIĆ LJ. (2003): The Present Situation in the Livestock Production in the Republic of Serbia .7 International Symposium, Modern Trends in Livestock Production, Biotechnology in Animal Husbadry, 19 (5-6), p.13-23.

PETROVIĆ,M. M. (2005): Livestock production in Serbia on way to European Union. Biotechnology in Animal Husbandry, 21, (5– 6), p.1 – 8.

PETROVIĆ, M.M., SRETENOVIĆ, LJ., PANTELIĆ, V., ALEKSIĆ, S., MIŠČEVIĆ, B., BOGDANOVIĆ, V., OSTOJIĆ-ANDRIĆ, D., PETROVIĆ, M. (2006): Results of the Aplication of the Tehnology of Genetic Improvement of Simmental Cattle Population in Serbia. Biotehnology in Animal Husbandry, 22(1-2), p.1 – 8.

PETROVIĆ M.M., ALEKSIĆ, S., SRETENOVIĆ Lj, MARINKOV G, STOJANOVIĆ LJ., TOMAŠEVIĆ D (2007): "The Effect of Genotype on Quality of Beef". Animal Science, British International journal of fundamental and applied research vol 1. 3. International conference, Quality and safety in meat for consumer: from stable to table, Caunas , Lithuania, 06-07 June, p.102-104.

PETROVIĆ M. M., PETROVIĆ M. P., M. PETROVIĆ, S. ALEKSIĆ, D. D. OSTOJIĆ-ANDRIĆ, V. PANTELIĆ, Ž. NOVAKOVIĆ (2011): How to increase

production of beef, lamb and pork in Serbia for domestic market and export. 3 rd International Congress-New Perspectives and Challenges of Sustainable Livestock Production, Belgrade, October 5-7. Biotechnology in Animal Husbandry, 27, 293-303..

PETROVIĆ M., PUŠIĆ M., RADOJKOVIĆ D., MIJATOVIĆ M., RADOVIĆ Č., ŽIVKOVIĆ B . (2006): Phenotypic and genetic variability of quality traits of carcass sides and meat**.** Biotechnology in Animal Husbandry, 22 (5-6):1-10.

PETROVIĆ M., RADOJKOVIĆ D., RADOVIĆ Č. (2012): Rezultati preduzimanih mera selekcije u svinjarstvu Srbije. Deseto savetovanje sa medjunarodnim učešćem "Zdravstvena zaštita, selekcija i reprodukcija svinja", Srebrno jezero, 31. maj- 02. jun, 2012. Zbornika radova, 7-13.

PETROVIĆ M.P., RUŽIĆ - MUSLIĆ, D., MAKSIMOVIĆ, N. (2009): Procena genetskog potencijala ovaca u različitim proizvodnim sistemima. Biotechnology in Animal Husbandry, vol. 25, 5-6-1, p. 421-429.

SIMM G., BÜNGER L., VILLANUEVA B., Hill W. G. (2004): Limits to yield of farm species: genetic improvement of livestock. In Yields of farmed species: constraints and opportunities in the 21st century. Nottingham, UK: Nottingham University Press., pp. 123–141.

SIMM, G. (1998): Genetic improvement of cattle and sheep. p. 1-433.

VOLLEMA A.R. (1998): Longevity of dairy cows: A review of genetic variances and covariances with conformation. Animal breeding abstracts 66 (9), p. 781.

***Statistical Office of Serbia, Belgrade: Census of Agriculture 2012 in the Republic of Serbia – First results

***Statistički godišnjak Republike Srbije (STAT.GOD.SRB.2012.): Republički zavod za statistiku, Beograd, 1985 - 2012.

Received 26 January 2013; accepted for publication 18 March 2013

Biotechnology in Animal Husbandry 29 (1), p 19-28 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 615.256:636.082'632
DOI: 10.2298/BAH1301019M

RESULTS OF APPLYING GONADORELIN FERTAGYL ON THE PROGESTERONE CONCENTRATIONS IN THE BLOOD SERUM AND COW CONCEPTION

N. Mlaćo[1], A. Katica[1], B. Čengić[2], V. Šakić[3]

[1] Veterinary Faculty, University of Sarajevo, Department for Histology and Embryology, Zmaja od Bosne 90, 71000 Sarajevo, BiH
[2] Veterinary Faculty, University of Sarajevo, Department for Obstetrics and Udder Diseases Zmaja od Bosne 90, 71000 Sarajevo, BiH
[3] Veterinary Faculty, University of Sarajevo, Department for Animal Husbandry, Zmaja od Bosne 90, 71000 Sarajevo, BiH
Corresponding author: nadzida.mlaco@vfs.unsa.ba
Original scientific paper

Abstract: Considering there are different and inconsistent results of the wider use of gonadotropin-releasing hormone or its analogs in cattle breeding and due to scarce research studies in the field of individual breeding, we decided to explore the influence of gonadorelin Fertagyl on the results of the blood serum progesterone levels and conception of cows in veterinary field practice. Examination of the progesterone levels involved 14 (fourteen) cows treated with gonadorelin (GnRH), Fertagyl, manufactured by Intervet, according to the manufacturer's instructions dose of 2,5ml (250 micrograms), within five minutes after insemination. There are individual differences in progesteron concentrations according to days of sampling in treated cows, respectively individuality is manifested in all cows between days of blood sampling, except for period of luteolysis (18th day) in non-gravid cows. Our research results indicate that there is a tendency for progesterone concentration to increase in gravid cows treated with Fertagyl at the time of insemination. Progesteron concentrations in blood serum in gravid cows were higher for 0.21 ng/ml in first three days after insemination and at 6th day those concentrations were higher for 0.40 ng/ml compared to non-gravid cows.

Key words: Fertagyl, progesterone, blood serum, cow

Introduction

Considering there are different and inconsistent results of the wider use of gonadotropin-releasing hormone or its analogs in farm breeding and due to scarce research studies in the field of individual breeding, we decided to explore the

influence of gonadorelin Fertagyl on the results of the blood serum progesterone levels and conception of cows in veterinary field practice. The blood of treated cows was sampled to examine the level of progesterone concentration in the serum of cows.

Materials and Methods

Examination of the progesterone levels concentration involved 14 (fourteen) cows treated with gonadorelin (GnRH), Fertagyl, manufactured by Intervet, according to the instructions provided by the manufacturer in dose of 2,5 ml (250 micrograms) and 14 (fourteen) cows treated with physiological solution (placebo). After taking the anamnestic data from the owners, such as the time of onset of estrus, and after performing gynecological examination of cows, Fertagyl was applied five minutes after insemination. The research included cows brought in for insemination for the first time, mostly 50-80 days after calving, and those that were re-entering estrus for the second or third time. Blood sampling was performed 3, 6, 9, 12, 15 and 18 days after insemination, that is, after the treatment with gonadorelin. The blood for determining the level of progesterone was taken by puncturing the tail vein with vacuum syringe. Following the blood sampling, serum was taken out and stored at -20 degrees C until the time of testing. Radioimmunoassay method (RIA) as well as commercial packages were used to determine the level of progesterone concentration. Radioactivity of samples was at all times measured under the same conditions using Gama Scintillation counter. The results were calculated according to the instructions provided by the reagent's manufacturer. The diagnosis of gestation was performed 50 to 60 days after insemination.

Results and Discussion

The levels of progesterone relative to the number of the serum samples from 14 (fourteen) cows show significant individual discrepancies in the level of progesterone concentration in relation to the days of sampling. The progesterone concentrations ranged from the minimum value of 0.80 ng/ml to the maximum value of 1.64 ng/ml in the first three days. On day 6 of sampling, there are also significant variations, however, of some lesser degree. In the majority of cows, except in cow 6 and 10, more equal elevation of the concentration level begins after day 6 until day 15.

Table 1. Levels of progesterone concentration (ng/ml) in the blood serum of gravid cows treated with GnRH (Fertagyl)

Number of cows	Blood sampling days after insemination					
	3	***6***	***9***	***12***	***15***	***18***
1	0.94	2.83	7.00	7.18	6.80	7.00
2	1.64	3.90	6.20	6.50	5.70	6.10
3	1.50	3.54	5.00	9.42	8.80	9.00
4	1.24	5.52	7.88	7.60	6.90	7.50
5	1.30	4.78	6.00	7.00	7.20	6.50
6	1.39	2.10	2.97	3.68	3.75	5.58
8	1.62	3.20	6.70	7.41	6.56	6.91
9	1.40	1.51	3.93	4.38	6.15	9.19
10	0.81	0.85	2.42	2.52	2.63	4.37
14	1.30	3.10	4.90	7.00	6.80	5.90
TOTAL	**13.14**	**31.33**	**53.00**	**62.69**	**61.29**	**70.05**
Average	1.31	3.13	5.30	6.26	6.13	7.00

The serum progesterone concentrations in the blood serum of gravid cows were higher by 0.21 ng/ml in the first three days after insemination, and on day 6, the concentrations were higher by 0.40 ng/ml in relation to non-gravid cows. In addition, there are large individual discrepancies in progesterone concentration in all gravid cows during all of the time periods of blood sampling. Initial concentrations of progesterone in some cows were very low all the time until the recognition of gravidity, when they started to elevate (cow 6 and 10).

Our results of the progesterone testing study also suggest there is a tendency for concentrations of progesterone to increase in gravid cows treated with Fertagyl at the time of insemination. There is appearance of an increasing concentration of progesterone in the blood of the cows treated with Fertagyl, which manifests on day 3 and 6 of blood sampling, as the individual discrepancy in progesterone concentration is very large during all sampling times.

Table 2. Levels of progesterone concentration (ng/ml) in the blood serum of non-gravid cows treated with gonadorelin (Fertagyl)

Number of cows	Blood sampling days after insemination					
	3	6	9	12	15	18
7	1.20	2.93	5.89	7.23	6.73	3.43
11	1.10	2.80	4.70	5.60	6.20	4.40
12	0.80	2.20	4.80	5.34	4.71	3.70
14	1.30	3.10	4.90	7.00	6.80	5.90
TOTAL	4.40	11.03	20.29	25.17	24.40	17.43
Average	1.10	2.75	5.07	6.29	6.11	4.35

As in gravid cows, there is also a gradual increase in concentration of progesterone in relation to the sampling days, except on day 18, when there is a significant decline due to the onset of luteolytic activity of prostaglandin from uterus.

Table 3. Levels of progesterone concentration (ng/ml) in the blood serum of gravid cows treated with placebo

Number of cows	Blood sampling days after insemination					
	3	6	9	12	15	18
1	1.30	2.33	5.08	5.13	6.71	7.96
4	1.37	3.56	3.98	4.76	5.06	7.95
5	0.04	0.15	4.50	5.60	5.06	5.40
8	1.10	2.76	3.96	4.52	4.47	4.97
9	1.20	2.30	4.50	5.67	5.76	6.16
10	1.52	2.02	2.04	2.35	4.26	6.02
11	0.72	1.42	4.00	6.10	6.30	6.00
12	1.20	2.30	4.40	6.50	5.90	5.20
TOTAL	**8.51**	**16.84**	**32.46**	**40.63**	**44.52**	**49.66**
Average	1.06	2.10	4.05	5.07	5.56	6.20

It is evident that the median concentrations of progesterone relative to the time of blood sampling are similar during the equivalent sampling periods, with quite a proper trend, except in cow 10, whose levels of the progesterone

concentration were low all the time until day 15. The levels of progesterone concentrations were also rather high on day 18 of sampling.

Table 4. Levels of progesterone concentration (ng/ml) in the blood serum of non-gravid cows treated with placebo

Number of cows	Blood sampling days after insemination					
	3	***6***	***9***	***12***	***15***	***18***
2	0.45	1.13	3.47	5.65	7.65	3.87
3	0.01	0.18	0.20	0.27	0.74	0.01
6	1.48	2.65	6.29	7.49	6.98	3.50
7	0.84	1.35	1.99	2.10	2.16	2.98
13	1.00	2.90	3.80	5.40	6.00	3.90
14	0.90	3.40	5.40	6.30	6.10	3.50
TOTAL	**4.68**	**11.61**	**21.15**	**27.21**	**29.63**	**17.76**
Average	0.78	1.93	3.52	4.53	4.97	2.96

Although, the average picture of progesterone secretion indicates the gradual period of elevation, maintenance and the degree of decline in hormonal secretion, the profiles of progesterone concentration indicate that there is individual variation between days of blood sampling, in fact, except for the period of luteolysis (day 18) in non-gravid cows, when there is a more significant decline in progesterone concentration. Very low concentrations of progesterone were observed in three cows (2, 4, and 7) all the time until day 9 after insemination.

Sexual cyclicity of cows is stimulated through the *hypothalamus-pituitary-ovary-uterus* mechanism. Ovulation comes at the end of estrus, as well as formation of the "yellow body" – *Corpus luteum* (plural *Corpora lutea*). Luteal cells of „yellow body" are glandular cells which produce steroids, respectively luteal cells excrete second female sexual hormon Progesteron. Formation of „yellow body" is under influence LH and excretion of Progesterone is under influence LTH (*Katica at al., 2010*). Secretion of progesterone stops abruptly several days before the next estrus. The period of *corpus luteum* activity represents the luteal phase that lasts 16 to 17 days in cows, while the follicular phase lasts from 3 to 6 days. The picture of the blood progesterone level in cows during the sexual cycle is well defined. The progesterone levels are at the minimum values around the time of estrus < 0.5 ng/ml; on day 4 after estrus, the concentrations are gradually starting to elevate up to the maximum values, from 4 to 13 ng/ml between day 8 and day 15. After that, the levels of progesterone concentrations drop down to the initial basal values, some two days before estrus (*Pope et al., 1969; Schams et al., 1989*). According to *Schamberger et al.,(1967*), the average

concentration of progesterone on days 6, 11, 16 and 21 is 3; 4, 8; 9.6; and 1.5 ng/ml. *Gupta and Pope*, (*1968*) published about cyclical level of progesterone concentrations in non-gravid cows. The progesterone concentrations in luteal phase ranged from 7.5 to 10 ng/ml and from 1 to 2 ng/ml around day 4 to day 6 before the ovulation. According to *McCraken* (*1991*), in two cows, during two consecutive sexual cycles, the average progesterone concentration in plasma on day 12 of the sexual cycle was 9.6 and 8.8 ng/ml.

Stabenfeldt et al., (1969) point out that the levels of progesterone vary from 0.4 ng/ml in the blood plasma at the time of estrus to approximately 7 ng/ml at the peak of the luteal phase of the sexual cycle (range from 6.1 to 10.2 ng/ml). The decline on day 18 continued until the next sexual cycle, and the first significant elevation in the next cycle occurred on day 4. According to *Garverick et al., (1971*), the progesterone levels are at the minimum value around estrus, < 0.5 ng/ml, on day 4 after estrus; the concentrations are gradually elevating, reaching the level of 4 to 13 ng/ml between day 8 and day 15 of the cycle, and after that, they drop down to the basal values some two days before next estrus cycle.

The progesterone concentrations in plasma during the first 14 days after estrus are similar regardless of whether the animal conceived or not (*Pope et al., 1969*), and after that, the values in non-gravid cows drop down; however, in gravid animals, the values are either maintained or elevated, staying at the level of around 9 ng/ml between day 30 and day 80. Studying the successfulness of the GnRH application in cows re-entering estrus in the field conditions in relation to the time intervals of the onset of estrus (6 to 8, 9 to 12 hours and longer), the results of conception were significantly better in treated cows in relation to controlling cows, if insemination and application of gonadorelin were performed within 6 to 8 hours after the first signs of estrus (77.41%:56.06%), (*Mutevelić et al., 2003*). The levels may fluctuate during the gravidity.

Ferizbegović (*1995*) points out that there are very significant individual discrepancies in the progesterone concentration levels in the serum of cows from highland and mountainous area. The discrepancies manifested particularly in relation to the time of duration of the first sexual cycles after calving, post partum. The author also observed that there were significant individual discrepancies in the maximum concentrations of progesterone in relation to the length of sexual cycles. The maximum concentrations of progesterone in short sexual cycles range from 1.92 to 3.77 ng/ml; in normal sexual cycles, they range from 3.58 to 8.10 ng/ml and from 2.50 to 7.10 ng/ml in long cycles.

Endogenous appearance of luteinizing hormone (LH) during estrus is significant and vital for ovulation and luteinization of granulosa and thecal cells, whose production of progesterone is necessary for maintaining of gestation (*Henderson 1979*). Injection of GnRH at the time of artificial insemination 10 hours after the first sign of estrus was able to induce additional appearance of LH (*Lee et al. 1985*). LH secretion and dynamics of follicular development after

application of gonadorelin, had been monitored by Marcelo et al., 2003 in their own researches.

The cows that responded to GnRH with median LH had larger production of progesterone than other groups. This was recorded as early as first four days after insemination. Hence, the higher progesterone in cows treated with GnRH, which were gravid, was likely due to appearance of LH after the application of GnRH, causing more of granulosa cells to become luteal cells for the production of progesterone or due to the improvement in the production of progesterone by the existing cells. Furthermore, it was announced that there was a significant relationship between the progesterone concentration and gravidity in heifers. The importance of progesterone concentration for maintaining gravidity was elaborated (*Lee et al., 1985*), which points out that 14% of cows in an early period post partum did not produce enough progesterone after ovulation to maintain gravidity, if there was conception. The progesterone concentrations during sexual cycle before ovulation may also be the indicator of later fertility in cows (*Fonesca et al., 1983*).

Our results prove that the treatment during the insemination of cows re-entering estrus may improve fertility, which is in relation with results of *Leslie and Kelton* (*1992*), which during research of gonadorelin (GnRH) application in the time of arteficial insemination in 93 dairy herd in Ontario, Canada, determined that there is improvement of conception in cows that three or more times need to be re-breed. It should not ignore the possibility that the percentage of conception is improved by repeatedly treating the same cow with Fertagyl during consecutive insemination. It was determined that repeat-breeder cows or heifers needed to be inseminated three or more times in order to successfully conceive and that they had normal interestrus interval and anatomic normal reproductive tract, (*Zobel at al., 2011*). The treatment with GnRH during estrus is likely to affect the time of ovulation, the fertility, the development of *corpus luteum*, the secretion of progesterone and the survival of embryo, (*Jadav at al., 2010*). In terms of conception it should not forget anti-luteolytic activity of bTP-1 (bovine throphoblast protein complex). The higher degree of conception in cows treated with GnRH is likely the result of an increased production of progesterone, which maintains gravidity. Treatment with GnRH during estrus probably influences, among other things, the development of *corpus luteum* and the secretion of progesterone.

Conclusions

- There is the tendency for progesterone concentrations to increase in the blood of gravid cows treated with Fertagyl, which manifests on day 3 and 6 of blood sampling, as the individual discrepancy in

concentrations of progesterone is very large during all sampling periods in both controlling and treated cows.

- A quantitative tendency of increasing concentrations of progesterone in the blood of gravid cows treated with Fertagyl is observed in gravid cows in relation to the sampling days, except on day 18, when there is a significant decline due to the onset of luteolytic activity of prostaglandin from uterus.
- The progesterone concentrations in plasma during the first 14 days after estrus are similar regardless of whether the animal conceived or not and after that, the values in non-gravid cows drop down; however, in gravid animals, the values are either maintained or elevated, staying at the level of around 9 ng/ml between day 30 and day 80.
- It seems that injection of GnRH at the time of artificial insemination (10 hours after the first sign of estrus) is able to induce additional secretion of LH.
- Cows that responded to GnRH with median LH had larger production of progesterone than other groups.
- The treatment with GnRH during estrus is likely to affect the time of ovulation, the fertility, the development of *corpus luteum*, the secretion of progesterone and the survival of embryo.
- The higher degree of conception in cows treated with GnRH is likely the result of an increased production of progesterone, which maintains gravidity.
- Treatment with gonadorelin during the insemination of cows re-entering estrus may improve fertility.

Rezultati aplikacije gonadorelina Fertagyl na koncentracije progesterona u krvnom serumu i koncepciju krava

N. Mlaćo, A. Katica, B. Čengić, V. Šakić

Rezime

Uzimajući u obzir postojanje različitih i protivrečnih rezultata u široj upotrebi gonadotropnog-oslobađajućeg hormona ili njegovih analoga u uzgoju goveda i usled oskudnih istraživanja na polju pojedinačnog uzgoja, odlučili smo istražiti uticaj gonadorelina Fertagyl na rezultate nivoa progesterona u krvnom serumu i koncepciju krava u veterinarskoj terenskoj praksi.

Pregled nivoa progesterona je uključivao 14 (četrnaest) krava tretiranih sa gonadorelinom (GnRH) Fertagyl, od proizvođača Intervet i to prema uputstvu u dozi od 2,5 ml (250 mikrograma), unutar 5 minuta nakon osemenjavanja. Postoje individualne razlike u koncentracijama progesterona u odnosu na dane uzorkovanja u tretiranih krava, odnosno individualnost se manifestuje u svih krava između dana uzorkovanja krvi, osim za period luteolize (18. dan) kod negravidnih krava.

Naša istraživanja ukazuju da postoji težnja da koncentracije progesterona porastu kod gravidnih krava tretiranih sa Fertagyl u vremenu osjemenjavanja. Koncentracije progesterona u serumu gravidnih krava bile su veće za 0,21 ng/ml u prva tri dana nakon osjemenjivanja, a 6. dana te koncentracije su bile više za 0,40ng/ml u odnosu na negravidne krave.

References

FERIZBEGOVIĆ J. (1995): Istraživanja sezonalnosti spolne cikličnosti krava brdsko-planinskog područja. Disertacija.

FONSECA F. A., BRITT J. H., McDANIEL B. T., WILK J.C., RAKES A. H. (1983): Reproductive traits of Holstein and Guernsey. Effects of age, milk, yield and clinical abnormalities on involution of cervix and uterus, ovulation, estrus cycles, detection of estrus, conception rate and day open. J. Dairy Sci. 66, 1128.

GARVERICK H.A., ERB R.E., NISWENDER G. D., THIMONIER J. (1971): Reproductive steroids in the bovine. III. Changes during the estrus cycle. J. Anim. Sa. 32., 946-956.

GUPTA S.K., POPE G.S. (1968): Variation in the level of progesterone in the systemic plasma of the cow. Endocrinology 40., XII.

HENDERSON K.M. (1979): Gonadotrophic regulation of ovarian activity. British Med. Bu. 35., 161-166.

JADAV, PV, PATEL, DM, KAVANI, FS., DHAMI, AJ. (2010): GnRH and its Applications in Bovine Reproduction. J. Adv. Dev. Res., Vol-1(1);74-80.

KATICA A., MLAĆO N., HASANBAŠIĆ D., HAMZIĆ E. (2010): Osnove veterinarske histologije.

LEE C.N., CRITSER J.K. and AX R.L. (1985): Changes of luteinizing hormone and progesterone for dairy cows after gonadotropin-releasing hormone at first postpartum breeding. J. Dairy Sci. 68., 1463-1470.

LESLIE K.E., KELTON D.F. (1992): The effect of Fertagyl administered at the time of breeding on fertility in lactating dairy cows. University Guelph.

MARCELO F. MARTINEZ, REUBEN J. MAPLETOFT, JOHN P. KASTELIC, TERRY CARRUTHERS. (2003): The effects of 3 gonadorelin products on luteinizing hormone release, ovulation, and follicular wave emergence in cattle. Can Vet J., 44(2); 125-131.

McCRACKEN J.A. (1963): Plasma progesterone concentration after removal of the corpus luteum in the cow. Nature, London 198. 507.
MUTEVELIĆ A., FERIZBEGOVIĆ J., MUTEVELIĆ T. (2003): Reprodukcija domaćih životinja.
POPE G.S., GUPTA S.K., MUNRO J.B. (1969): Progesterone levels in the systemic plasma of pregnant, cycling and ovariectomized cows. J. Reprod. Fertil. 20. 369-381.
SCHAMBERGER D.W., CONDERT S.P., SHORT R.V. (1967): Effects of bovine luteinizing hormone and human chorionic gonadotropin on the bovine corpus luteum in vivo. J. Reprod. Fertil. 14. 277.
STABENFELDT G.H., EWING L.L., McDONALD L.E. (1969): Peripheral plasma progesterone levels during the bovine estrus cycle. J. Reprod. Fertil. 19. 433-442.
ZOBEL R., TKALČIĆ S., BUIĆ V., PIPAL I., GEREŠ D., SAMARDŽIJA M. (2011): Repeat breeder syndrome in dairy cows:influence of breed and age on its prevalence and the success of hormone therapy. Turk. J. Vet. Anim. Sci.; 35(6); 405-411 © TÜBITAK doi 10.3906/vet-1001-236.

Received 16 January 2013; accepted for publication 15 March 2013

Biotechnology in Animal Husbandry 29 (1), p 29-36 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.06'636.237
DOI: 10.2298/BAH1301029D

METABOLIC STATUS IN SIMMENTAL DAIRY COWS DURING TRANSITION PERIOD

R. Đoković[1] , V. Kurćubić[1], Z. Ilić[2], M. D. Petrović[1], J. Stojković[2], B. Milošević[2], M. Cincović[3]

[1]Department of Animal Science, Faculty of Agronomy-Čačak, University of Kragujevac, Cara Dušana 34, Čačak.
[2]Department of Animal Science, Faculty of Agronomy, University of Priština, Kopaonička bb. Lešak,
[3]Department of Veterinary Medicine Faculty of Agriculture, University of Novi Sad, Trg D.Obradovića 8, Novi Sad.
Corresponding author: djokovici@ptt.rs
Original scientific paper

Abstract: The objective of the present study was to determine metabolic status in late pregnant (n = 15) and puerperal (n = 15) Simmental dairy cows. The various blood metabolites and serum enzyme activities were determined by photometric methods. The early lactation cows had the indicative values of the beta-hydroxybutyrate (BHB) (> 1.20 mmol/l) but did not display any clinical signs, which means that they had a typical subclinical condition. The lipomobilization markers, serum BHB and non-esterified fatty acids (NEFA) concentrations, were markedly enhanced ($P<0.05$) in early lactation cows. Liver steatosis compromised hepatocyte metabolism, leading to significantly weaker ($P<0.05$) circulating concentrations of glucose, triglyceride (TG) and urea, and induced some cellular lesions as evidenced by significant increases ($P<0.05$) in the serum bilirubin concentrations and theaspartate transaminase (AST) enzyme activities in early lactation cows. On the basis biochemistry estimation, early lactation cows had metabolic disturbances which were associated with ketosis, and some degree of hepatic lesions, probably due to fat infiltration.

Key words: dairy cows, transition period, liver steatosis, ketosis, blood metabolites, enzymes.

Introduction

Transitional period in dairy cows included 3 weeks before and 3 weeks after calving when metabolic processes were adapted to providing energy and nutrients required for synthesis of milk compounds (*Overton and Waldron, 2004*). Major health disorders in high-yielding cows occur around parturition. They include sudden changes in energy metabolism that can induce severe uncontrolled

disorders related to the organic matter metabolism (*Drackley et al., 2005*). As a consequence, such a state caused negative energy balance, a high mobilization of lipids from body fat reserves as well as hypoglycaemia in early lactation (*Djoković et al., 200;, Civlelek et al., 201;, Gonzales et al., 2011*). Lipomobilisation characterized by high blood non-esterified fatty acids (NEFA) concentrations starts within high pregnancy and reaches a maximal intensity in the early lactation (*Veenhuizen et al., 1991; Vazquez-Anon et al., 1994; Dann et al., 2005; Djoković et al., 2007*). NEFA are preferentially and greatly accumulated as triglyceride (TG) in the liver, primarily because of a decrease in the very low density lipoproteins (VLDL) synthesis by hepatocytes (*Herd et al., 1983; Jorritsma et al., 2001; Sevinc et al., 2003*). Consequently, physiological situations leading to a negative energy balance (fasting, parturition and lactation) are coupled to an increased uncontrolled rate of body fat mobilisation and the increased fatty acids accumulation in hepatocytes, resulting in disturbances of the morphological and physiological liver integrity (*Veenhuizen et al., 1991; Vazquez-Anon et al., 1994; Djokovic et al., 2007*). However, when an important steatosis occurs, the endogenous liver syntheses are lowered leading to decreases in blood concentrations of glucose, total proteins (TP), albumin and globulins, cholesterol, TG and urea. Furthermore, the excretory function of hepatocytes is reduced and accordingly, the blood concentrations of some compounds such as total bilirubin, ammonia and bile acids are generally increased (*West, 1990; Herd et al., 1983; Sevinc et al., 2003; Bobe et al., 2004; Drackley et al., 2005*). The fatty liver infiltration and the hepatocyte degeneration involve cell membrane damage and hepatocyte destruction coupled to the release of cytoplasm enzymes (AST, GGT, LDH) and marked increases in the circulating activities (*Pechova et al.,1997, Lubojacka et al.,2005*).

The objective of the present study was to determine metabolic status in transitional dairy cows on the basis of blood concentrations of various metabolites.

Materials and Methods

This experiment was carried out in the January 2012 in dairy herd (119 Simmental cows) with several metabolic and reproductive disorders (Farms: Ćurcić, Mrsać, Kraljevo). The cows were mid-yielding with a preceding lactation about 6.500 l (late pregnant cows - 6392 ± 1005 l and early lactation cows 6488 ± 980 l in previous lactation). Two groups of clinically healthy cows were chosen from herd. One group consisted of late pregnant cows (n = 15) in period from 25 to 1 (13.7 ± 9.3) days to partus and a second group included early postpartum cows (n = 15) in the first month of lactation (16.1 ± 9.2 days). The estimated cows had body score condition among 3.5 and 4.0. The experimental cows were kept in tie-stall barns. The diet and the housing facilities were adapted to research purposes. The diet suited the energy necessary for cows in late pregnancy and early lactation.

The cows in late pregnancy were fed with a diet consisting of 6 kg lucerne hay, 15 kg maize silage (30% dry mater,DM) and 3 kg concentrate (30% crude proteins, CP). The cows in early lactation were fed with a diet consisting of 7 kg lucerne hay, 20 kg maize silage (30% DM) and 5 kg concentrate (30% CP). Dietary nutrient contents for dairy cows in late pregnancy and in early lactation are given in Table 1.

Table 1. Nutrient contents in daily ration for dairy cows in the late pregnancy and in the early lactation

	Late pregnancy	Early lactation
Dry Matter (DM) (kg)	11.94	16.05
Net Energy of lactation (NEL) (MJ)	65.25	87.15
Crude Protein (CP) (% of DM)	12.55	13.58
Rumen undegradable protein (RUP) (% of CP)	30.86	35.91

The 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 according to the same modalities and plasmas were assessed for glucose concentrations. The beta-hydroxybutyrate (BHB), non-esterified fatty acids (NEFA), triglyceride (TG), glucose, total proteins (TP), albumin, urea, total bilirubin and serum aspartate transaminase (AST) and gamma-glutamyl transferase (GGT) were measured in the biochemical laboratory Kvarklab. (Kragujevac, Serbia) by different colorimetric techniques using a spectrophotometers (Cobas Mira and Gilford Stasar) and the corresponding commercial kits.

The statistical analysis of the obtained data was carried out by ANOVA-procedure (Statgraphic Centurion, Statpoint Technologies Inc.Warrenton, Va). 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

The present study compared the metabolic status in dairy cows during transition period. The results of the selected blood metabolites in cows in the transition period and correlations among blood metabolites are given in Tables 2 and 3.

Table 2. Blood metabolites in transitional dairy cows (n=15 in each group). Results are expressed as mean standard ± deviation.

Parameter	Late pregnant cows	Early lactation cows	P
Glucose (mmol/L)	3.36 ± 0.30	2.29 ± 0.48	< 0.05
BHB(mmol/L)	1.14 ± 0.36	1.59 ± 0.25	< 0.05
NEFA(mmol/L)	0.17 ± 0.06	0.38 ± 0.29	< 0.05
TG(mmol/L)	0.29 ± 0.07	0.12 ± 0.02	< 0.05
TP(g/L)	77.08 ± 4.57	78.89 ± 4.92	NS
Albumin(g/L)	42.57 ± 7.53	34.61 ± 3.56	< 0.05
Urea (mmol/L)	5.29 ± 1.32	3.60 ± 1.07	< 0.05
Total bilirubin (μmol/L)	3.26 ± 0.49	3.91 ± 2.85	NS
AST (IU/L)	33.55 ± 9.38	69.46 ± 30.89	< 0.05
GGT (IU/L)	20.61 ± 4.16	25.05 ± 4.91	NS

Legend:NS: not significant

Table 3. Correlation coefficients for the biochemical metabolites calculated for all cows in the present study. Significant correlations (P<0.05) are indicated with *

	NEFA	BHB	TG	TP	Albumin	Urea	Bilirubin	AST	GGT
Glucose	r= -0.35*	r=-0.47*	r=0.65*	r=0.01	r=0.47*	r=0.43*	r=-0.03	r=-0.23	r=-0.32*
NEFA		r=0.39	r=-0.21	r =-0.34*	r=-0.26	r =-0.45*	r= 0.63*	r= 0.34*	r=-0.17
BHB			r=-0.36*	r=-0.06	r=-0.23	r=-0.27	r=0.13	r=0.15	r=0.06
TG				r=0.05	r=0.63*	r=-0.61*	r=-0.28	r=-0.04	r=0.24
TP					r=0.11	r=-0.29	r=0.24	r=0.30	r=0.07
Albumin						r=-0.46*	r=-0.28	r=-0.29	r=-0.35*
Urea							r=-0.07	r=-0.33*	r=-0.14
Bilirubin								r=0.16	r=0.01
AST									r=0.22

The blood glucose values in the late pregnant cows were within physiological range 2.5 - 4.2 mmol/L (*Radostis et al., 2000*), whereas in early lactation cows hypoglycemia was determined. Nevertheless, glycaemia was significantly depressed (P<0.05) in puerperal cows compared to pregnant cows. This decrease in the glucose concentrations previously reported in different studies (*Veenhuizen et al., 1991, Drackley et al., 2001, Djokovic et al., 2007*) may be related to the sudden activity of the mammary gland and the increased lactose synthesis. In such situations, the serum BHB concentration is another indicator of energy metabolism disruptions which is more sensitive than glycaemia and which fluctuates in parallel to lipomobilization (*Civlelek et al., 2011, Gonzales et al., 2011*). In the present study, the lactating cows exhibited significantly higher

(P<0.05) BHB concentrations than the pregnant cows, suggesting a strong mobilisation of fat stores. Subclinical ketosis 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 (*Oetzel, 2004*). The early lactation cows had the indicative values of the BHB (1.59±0.25mmol/l) but did not display any clinical signs, which means that they had a typical subclinical condition. In the same way, the blood concentration of NEFA, considered as the best indicator of negative energy balance and of the lipomobilization intensity during the transition period (*Oetzel, 2004, Civlelek and al., 2011, Gonzales et al., 2011*) was also significantly increased (P<0.05) in the group of cows in early lactation compared to the group of late pregnant cows. Additionally, blood BHB and NEFA concentrations were found highly and positively correlated (r=0.39, P<0.05) together in the current study. The serum BHB and NEFA concentrations in puerperal cows clearly indicated that the intense lipomobilization in the post-partum period has induced ketogenesis and lipid overloading in the liver.

On the other hand, it was observed significant decreases (P<0.05) in the serum TG, urea and albumin concentrations in puerperal cows compared to the late pregnant females and TP were also decreased, although not significantly (P>0.05), during the post-partum period. In addition, all these biochemical parameters positively and some of them significantly (P<0.05) correlated together and with the glycaemia but were negatively correlated with the BHB and NEFA concentrations (Table 3). These results suggested an increased accumulation of TG in hepatocytes in the puerperal cows, probably linked to a depleted liver synthesis of VLDLs as previously evoked (*Herd et al.,1983; Jorritsma et al., 2001; Sevinc et al., 2003*). In the same way, the uraemia, proteinemia and albuminemia were lowered in puerperal cows compared to the late pregnant females, confirming the reduction of the liver syntheses induced by the development of fatty infiltration in liver (*West, 1990, Herd et al., 1983; Sevinc et al.,2003; Bobe et al., 2004; Drackley et al., 2005*).

By contrast, liver damage induces an increase in the serum total bilirubin, and the hemic compound is considered as a sensitive indicator for liver injury (15, 18). *West (1990)* reported a positive and significant correlation between the lipid amounts in the liver and the serum total bilirubin concentrations. In the same way, bilirubin concentrations significantly and positively correlated (r=0.63; P<0.05) with the NEFA concentrations here. In addition, the mean bilirubin concentration was significantly and markedly increased (P<0.05) in the puerperal cows compared to the late pregnant ones. As bilirubin concentrations, high serum activities of some enzymes highly expressed in liver in ruminants such as AST and GGT are observed in liver injury and highly contribute to evaluate the degree of tissue damage (*Pechova a et al.,1997; Lubojacka et al.,2005*). In the present study, the serum AST activities were significantly higher (P<0.05), and GGT activities were higher, but without significant difference (P>0.05) in early lactation cows,

corroborating that the development of fatty infiltration in liver has lead to cell disruption and release of the intracellular enzymes into the blood flow. Moreover, according to *Pechova et al. (1997),* the blood activities of liver enzymes are correlated with the degree of fatty infiltration in the organ. A positive correlation between AST activity and lipomobilization (NEFA values) was observed by the significant coefficient ($r= 0.34$; $P<0.05$). In the present study, all data concerning liver enzymes suggested that the process of lipomobilization was enough to cause liver lesions in the early lactating cows.

Conclusion

- This investigation demonstrated that the early lactation cows had the indicative values of the BHB (>1.20 mmol/l) but did not display any clinical signs, which means that they had a typical subclinical condition.
- The lipomobilization markers, serum BHB and NEFA concentrations, were markedly enhanced in early lactation cows. Liver steatosis compromised hepatocyte metabolism, leading to significantly weaker ($P<0.05$) circulating concentrations of glucose, TG and urea, and induced some cellular lesions as evidenced by significant increases ($P<0.05$) in the serum bilirubin concentrations and the AST enzyme activities in early lactation cows.
- On the basis of biochemistry estimation, early lactation cows had metabolic disturbances which were associated with ketosis, and some degree of hepatic lesions, probably due to fat infiltration.

Acknowledgment

This work was financed by Ministry of Education and Science, Republic of Serbia, projects TR. 31001.

Metabolički status mlečnih krava simentalske rase za vreme tranzicionog perioda

R. Đoković, V. Kurćubić, Z. Ilić, M. D. Petrović, J. Stojković, B. Milošević, M. Cincović

Rezime

Cilj ovog rada je bio da se proceni metabolički status kod visoko gravidnih mlečnih krava (n=15) i mlečnih krava na početku laktacije (n=15) Simentalske rase. Metaboliti i enzimska aktivnost krvnog seruma su određivani fotometriskom metodom. Krave na početku laktacije su imale indikativan nivo beta-hidroksi buterne kiseline (BHB) (>1.20 mmol/l) u krvi, karakteristčnu za subkliničku ketozu. Markeri lipomobilizacije, beta-hidroksi buterna kiselina (BHB) i neesterifikovane masne kiseline (NEFA), bili su statističko značajno veći ($P<0.05$) kod krava na početku laktacije u odnosu na visoko gravidne krave. Masna infiltacija ćelija jetre uzrokuje značajno nižu ($P<0.05$) vrednost glukoze, triglicerida, albumina i ureje u krvi, kao i ćeliska oštećenja koje se manifestuju značajnim povećanjem ($P<0.05$) koncentracije ukupnog bilirubina i aktivnosti aspartat-transaminaze (AST) u krvnom serumu kod krava na početku laktacije. Na osnovu rezultata biohemijskih ispitivanja može se zaključiti da kod krava na početku laktacije postoje metabolički poremećaji koju su povezani sa ketozom, kao i oštećenja hepatocita koji su verovatno nastali kao posledica masne infiltracije ćelija jetre.

References

BOBE G., YOUNG J.W., BEITZ D.C. (2004): Pathology, etiology, prevention, treatment of fatty liver in dairy cows. Journal of Dairy Science, 87, 3105-3124.

CIVELEK T., AYDIM I., CINGI C.C., YILMAZ O., KABU M. (2011): Serum non-esterified fatty acids and beta-hydroxybutyrate in dairy cows with retained placenta. Pakistan Veterinary Journal, 31(4), 341-344.

DANN H.M., MORIN D.E., MURPHY M.R., BOLLEROG A., DRACKELY J.K. (2005): Prepartum intake, postpartum induction of ketosis, and periparturient disorders affect the metabolic status of dairy cows. Journal of Dairy Science, 88, 3249-3264.

DJOKOVIĆ R., ŠAMANC H., JOVANOVIĆ M., BOŠKOVIĆ-BOGOSAVLJEVIĆ S. (2007): Changes in blood values of glucose, insulin and inorganic phosphorus in healthy and ketotic cows after intravenous infusion of propionate solution. Acta Veterinaria Brno, 76, 533-539.

DRACKELY J.K., OVERTON T.R., DOUGLAS G.N. (2001): Adaptations of glucose and long-chain fatty acid metabolism in liver of dairy cows during the periparturient period. Journal of Dairy Science, 84(E. Suppl.), p100-p112.

DRACKELY J.K., DANN H.M., DOUGLAS G.N., JANOVICK GURTZKY N.A., LITHERLAND N.B., UNDERWOOD J.P. LOOR J.J. (2005): Physiological and pathological adaptations in dairy cows that may increase susceptibility to periparturient diseases and disorders. Italian Journal of Animal Science, 4, 323-344.

GONZALES F.D., MUINO R., PEREIRA V., CAMPOS R. (2011): Relationship among blood indicators of lipomobilization and hepatic function during early lactation in high-yielding dairy cows. Journal of Veterinary Science, 12 (3), 251–255.
HERDT T.H., LEISMAN J.S., GERLOFF B.J., EMERZY R.S. (1983): Reduction of serum triacilglycerol-rich lipoprotein concentrations in cows with hepatic lipidosis. American Journal Veterinary Research, 44, 293-296.
JORRITSMA R.H., JORRITSMA Y.H., SCHUKKEN P.C., BARTLETT T., WENSING T., WENTING G. (2001): Prevalence and indicators of postpartum fatty infiltration of the liver in nine commercial dairy herds in the Netherlands. Livestock Production Science, 68, 53-60.
LUBOJACKA V., PECHOVA A., DVORAK R., DRASTICH P., KUMMER V. POUL J. (2005): Liver steatosis following supplementation with fat in dairy cows diets. Acta VeterinariaBrno, 74, 217-224.
OETZEL G.R. (2004): Monitoring and testing dairy herds for metabolic disease. Veterinary Clinics of North America: Food Animal Practice, 20, 651-674.
OVERTON T.R., WALDRON M.R. (2004): Nutritional management of transition dairy cows: Strategies to optimize metabolic health. Journal of Dairy Science, 87, E105-E119.
PECHOVA A., LLEK J., HALOUZKA R. (1997): Diagnosis and control of the development of hepatic lipidosis in dairy cows in the peri-parturient period. Acta Veterinaria Brno, 66, 235-243.
RADOSTIS. M., BLOOD D.C., GAY C.C., HINCHCCLIFF K.W. (2000): Veterinary Medicine, A Textbook of the Diseases of Cattle, Sheep, Pigs, Goats and Horses. Ninth Edition W.B. Saunders Company Ltd London New York Philadelphia San Francisco St. Louis Sydney
SEVENIC M., BASOGLU A. GUZBLBEKTA H. (2003): Lipid and lipoprotein levels in dairy cows with fatty liver. Turkish Journal of Veterinary Animal Science, 27, 295-299.
VAZQUEZ-ANTON M., BERTRICS S., LUCK M. GRUMMER R. (1994): Peri-partum liver triglyceride and plasma metabolites in dairy cows. Journal of Dairy Science, 77, 1521-1528.
VEENHUIZEN J.J., DRACKLEY J.K., RICHARD M.J., SANDERSON T.P., MILLER L.D., JOUNG J.W. (1991): Metabolic changes in blood and liver during development and early treatment of experimental fatty liver and ketosis in cows. Journal of Dairy Science, 74, 4238-4253.
WEST H.J. (1990): Effect on liver function of acetonaemia and the fat cow syndrome in cattle. Research inVererinary Science, 48, 221-227.

Received 1 August 2012; accepted for publication 18 December 2012

Biotechnology in Animal Husbandry 29 (1), p 37-43 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC615:616.24'62
DOI: 10.2298/BAH1301037K

BOVINE RESPIRATORY DISEASE COMPLEX (BRDC): VIRAL AND BACTERIAL PATHOGENS IN SERBIA

V. Kurćubić[1], R. Đoković[1], D. Vidanović[2], M. Šekler[2], K.Matović[2], Z. Ilić[3], J. Stojković[3]

[1]Faculty of Agronomy in Čačak, University in Kragujevac, Cara Dušana 34, 32000 Čačak, Republic of Serbia
[2]Veterinary Specialist Institute "Kraljevo", Žička 34, 36000 Kraljevo, Republic of Serbia
[3]Department of Animal Science, Faculty of Agriculture, University of Priština - Zubin Potok, Jelene Anžujske bb, Serbia.
Corresponding author: vladevet@tfc.kg.ac.rs
Original scientific paper

Abstract: Pathogens causing BRDC in Serbia were investigated. Two herds of beef cattle with bovine respiratory disease were included, with twenty diseased calves (10 from each farm) were chosen for isolation of bacteria on artificial culture media and determination by aerobic cultivation. The most common bacterial pathogen was isolated was *Pasteurella multocida*. Diffusion method of sensitivity to antibiotics (antibiogram), revealed that Enrofloxacin and Floron were most efficient antibiotics against *Pasteurella multocida* isolates (100 % isolates sensitive on both antibiotics). From the all examined samples (n=20) using the method of Real Time PCR (RT-PCR and PCR) we determined the genome sequences of bovine respiratory syncytial virus (BRSV), but in none of the samples genome of bovine viral diarrhea virus (BVDV) and bovine herpesvirus-1 (BoHV-1).

Key words: bronchopneumonia, viruses, bacteria, Real Time PCR, isolation, sensitivity on antibiotics.

Introduction

Bovine respiratory disease (BRDC) is a major disease problem for the cattle industry, and most costly disease with big economic losses: decreased production, higher levels of mortality and morbidity, increased labour costs and reduced carcass value *(Irsik et al., 2006)*. BRDC is multifactorial process, infectious agents including viruses, bacteria and mycoplasma *(Pardon et al., 2011; Duff et al., 2007; Ellis, 2001)*. Predisposing co-factors in the development of disease are: stress and environmental factors (weaning, temperature, stocking density, dust, humidity and shipping) and nutritional change *(Taylor et al. 2010; Snowder et al., 2006)*. Viral pathogens that causing primarily respiratory lessions are Bovine herpesvirus 1 (BoHV-1), Infectious bovine rhinotracheitis virus (IBRV), Bovine respiratory

syncitial virus (BRSV) and bovine parainfluenza Virus type 3 (BPI3V) *(Pardon et al., 2011; Lazić et al., 2009; Ellis, 2001)*. Unique among the bovine respiratory viral agents is BVDV, because intrauterine infection can lead in persistently infected (PI) cattle, chronically ill or dying in feedlots *(Loneragan et al., 2005; Kurćubić et al., 2011)*. Infection with BVDV lead to immunosuppression which causing progression of BRDC because facilitate invasion with opportunistic secondary pathogens such as *Mannheimia haemolytica* (16 serotypes), *Pasteurella multocida, Haemophilus somni* and a number of *mycoplasma* species such as *M. bovis* and *M. dispar (Pardon et al., 2011; Fulton, 2009; Hodgson et al., 2005; Ellis, 2001)*.

Molecular methods for identifying and sequencing the genomes of animal viruses are constantly updated, like developing of multiplex reverse transcription quantitative polymerase chain reaction (mRT-qPCR) assay (sensitive and specific technique capable of detection of three major viral respiratory pathogens of cattle by *Thonur et al., 2012*). Reagents for PCR assays are traditionally considered expensive, the ability to perform these assays within a short time frame to detect multiple pathogens can generate valuable information in differential diagnosis. Additional cost benefits on farm will result from more rapid diagnosis and the ability to target treatment, use appropriate vaccines or implement improved management procedures quickly. Molecular tests allow the assessment of development trends of microorganisms, a retrospective analysis of their geographical distribution and development of a database.

The aim of this study was to obtain basic knowledge of pathogens that cause BRDC in Serbia.

Materials and methods

Samples

Beef cattle, 5 months old, both sexes, Simmental race. Experimental animals are the property of "Kotlenik promet" d.o.o. Lađevci, from two farms, located near Čačak. From anamnesis and clinical examination on the day of sampling, we diagnosed the severe symptoms which justify suspicion of BRDC. Those symptoms were include: lose weight following a loss of appetite, visibly rapid breathing, lung auscultation tightened breathing, cough progresses to sound relatively dry, ocular and nasal discharge visible as either serous or yellow and viscous, depression and a progressive fever - rectal temperature above 40.1 ^{0}C.

Samples of discharge from the nasal mucosa were taken using sterile swabs and test tubes for the isolation of the etiological agents of viral and bacterial origin, from clinically dieseased and health animals in same herd (facilities). All samples were shipped in a hand portable refrigerator within one hour after sampling to the acredited microbiological laboratory of Veterinary Specialist Institute "Kraljevo".

Isolation of bacterial organisms on artificial culture media was determined by aerobic cultivation, with subsequent biochemical identification and determination of the isolated strains (*Dujin et al., 1984*).

Determination of bacteria presence

Sensitivity testing of isolated bacterial strains to antibiotics and sulfonamides (antibiogram) was performed using the disk diffusion method, according to Kirby-Bauer procedure (1966).

Real Time PCR (RT-PCR) method

a) Determining the presence of genome of IBRV

The genome of the virus IBRV was extracted using the QIAamp DNA Mini Kit (Qiagen, Germany). The real-time PCR reaction was performed using the Maxima™ Probe qPCR Master Mix (Fermentas, Lithuania), the Real Time PCR machine MX3000P Strategene, according to the protocol described in the OIE Manual, Chapter 2.4.13. *(OIE, 2008)*.

b) Determination of the BVDV and BRSV presence

BVDV genome was extracted using the QIAamp Viral RNA Mini Kit (Qiagen, Germany). Real time RT-qPCR reaction was performed using the Superscript III Platinum® One-Step Quantitative RT-PCR System (Invitrogen, USA), the Real Time PCR machine MX3000P Strategene. BVDV genomic RNA presence was done according to the protocol described by *Baxi et al. (2006)*, and detection and quantitation of BRSV was done with the protocol described by *Boxus et al. (2005)*.

Results and Discussion

Results of the microbiological tests and susceptibility testing are presented in the tables below (1-2).

From 10 swabs taken form nasal mucosa of beef cattle on farm 1, five animals (50 %) were positive on bacteriological examination (aerobically). Presence of the agents in positive beef cattle is as follows: *Pasteurella multocida* (5/50 %); *Aeromonas viridans* (4/40 %); *Corynebacterium bovis* (3/30 %); *Micrococcus luteus* (2/20 %); *Mannheimia haemolytica* (1/10 %). On farm 2, six animals (60 %) were positive on bacteriological examination (aerobically). Presence of the agents in positive beef cattle is as follows: *Pasteurella multocida* (6/60 %); *Corynebacterium bovis* (4/40 %); *Aeromonas viridans* (3/30 %); *Mannheimia haemolytica* (2/20 %); *Micrococcus luteus* (1/10 %).

Determination of the infectious bovine rhinotracheitis virus (IBRV) and BVDV genome presence revealed that the presence is not established, in all 20 examined samples (animals), from both farms. Genome of the BRSV is determined in all 20 examined samples, from both farms, and confirmed the findings of *Brodersen (2010)* that the BRSV is a major cause of respiratory disease and a major

contributor to the BRDC. Our result is in accordance to the observed clinical signs in diseased beef cattle.

Predominant isolated bacteria from nasal swabs were *Pasteurella multocida*, and the results of the isolates sensitivity to antibiotics and sulfonamides (antibiogram) is showen in table 2.From data presented in table 2, we can conclude that the most efficient antibiotics against *Pasteurella multocida* isolates were Enrofloxacin and Floron (11/100 % isolates sensitive on both antibiotics).

Table 1. Pathogens detected in the samples of nasal swab from beef cattle (FARM 1 and 2)

Values																				
	No. of samples (Farm 1)										No. of samples (Farm 2)									
Parameter	1013	8979	7102	355	1152	7137	444	305	7119	58	8380	9478	603	6721	9171	9923	8994	17007	7756	9829
Bacteriological examination (aerobically)	+	-	-	+	-	-	-	+	+	+	-	+	-	+	+	+	+	-	-	+
Mannheimia haemolytica	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	+	-	-	+
Pasteurella multocida	+	-	-	+	-	-	-	+	+	+	-	+	-	+	+	+	+	-	-	+
Aeromonas viridans	+	-	-	+	-	-	-	+	-	+	-	+	-	-	-	+	+	-	-	-
Micrococcus luteus	+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	+
Corynebacterium bovis	-	-	-	-	-	-	-	+	+	+	-	+	-	-	+	+	+	-	-	-
IBR/IPV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BVDV	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
BRSV	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

Table 2. *Pasteurella multocida* isolates sensitivity to antibiotics and sulfonamides (antibiogram)

Antibiotics	Farm 1					Farm 2					
	IS 1*	IS 2	IS 3	IS 4	IS 5	IS 6	IS 7	IS 8	IS 9	IS 10	IS 11
Amoxicillin	S	R	R	S	S	S	S	S	S	S	S
Ampicillin	S	R	R	S	S	S	S	S	S	S	S
Enrofloxacin	S	S	S	S	S	S	S	S	S	S	S
Gentamycin	I	R	S	I	S	I	R	I	I	I	I
Neomycin	S	S	S	S	S	-	-	-	-	-	-
Penicillin	I	R	R	S	S	-	-	-	-	-	-
Tetracycline	I	R	R	S	I	I	I	S	S	S	S
Trimetoprim+sulphometoxasol	I	R	R	S	S	I	R	I	S	S	I
Floron	S	S	S	S	S	S	S	S	S	S	S
Tylosin	-	-	-	-	-	I	I	I	I	I	I

Legend:IS 1* - no. of isolates; S - Sensitive; I - Intermediate sensitivity; R - Resitant.

Conclusion

The most common bacterial findings were *P. multocida*, *Aeromonas viridans* and *Corynebacterium bovis*, suggesting on their higher importance in BRDC in Serbian beef cattle with regard to *Mannheimia haemolytica*, predominantly determined worldwide. How these three pathogens interact together and with viruses remains to be clarified. According to Real-time RT-PCR and PCR findings BRSV is common virus present in Serbian beef cattle herds suffering from BRDC (BoHV-1 and BVDV genome were not identified in our study). Enrofloxacin and Floron were found to be the most efficient antibiotics against *P. multocida* isolates were 100 % of examined isolates sensitive on both antibiotics).

Acknowledgement

This research was financially supported by the Ministry of Education, Science and Technological Development, Republic of Serbia, TR 31001 project.

Kompleks respiratornog oboljenja goveda: virusni i bakterijski uzročnici u Srbiji

V. Kurćubić, R. Đoković, D. Vidanović, M. Šekler, K. Matović, Z. Ilić, J. Stojković

Rezime

Ispitivani su patogeni koji izazivaju BRDC u Srbiji. Iz dva stada tovne junadi smo odabrali dvadeset junadi obolelih od BRDC (po 10 sa svake farme) od kojih smo uzeli uzorke za izolaciju bakterija na veštačkim podlogama i određivanje aerobnom kultivacijom. Najčešće izolovana bakterija je bila *Pasteurella multocida*. Testiranje osetljivosti izolovanih bakterijskih sojeva na antibiotike i sulfonamide je realizovano difuzionom metodom (antibiogram), a najefikasniji antibiotici protiv izolata *Pasteurella multocida* su Enrofloxacin i Floron (100% izolata osetljivih na oba antibiotika). Od svih ispitivanih uzoraka (n = 20) sa obe farme metodom Real Time PCR (RT-PCR and PCR), ustanovili smo sekvence genoma bovinog respiratornog sincicijelnog virusa (BRSV), ali ni u jednom od uzoraka genom virusa bovine virusne dijareje (BVDV) i herpesvirusa-1 goveda (BoHV-1).

References

BAXI M., McRAEA D., BAXI S, GREISER-WILKE I., VILICEK S., KINGSLEY A., DEREGT D. (2006): A one-step multiplex real-time RT-PCR for detection and typing of bovine viral diarrhea viruses. Vet. Mic. 116 (1-3); 37-44.

BOXUS M., LETELLIER, KERKHOFS P. (2005): Real-time RT-PCR for the detection and quantitation of bovine respiratory syncicyal virus. J Virol Methods 125; 125-130.
BRODERSEN B.W. (2010): Bovine Respi ratory Syncytial Virus. Vet Clin Food Anim 26; 323-333.
DUFF G.C., GALYEAN M.L. (2007): Board-invited review: recent advances in management of highly stressed, newly received feedyard cattle. J. Anim. Sci. 85; 823-840.
DUJIN T., MARKOVIC B., MIHAJLOVIC B., S ŠIBALIC (EDS): Manual for the laboratory diagnosis - standardization of diagnostic methods for bacterial, viral and parasitic diseases of animals whose suppression is required by law. Committee for Publishing, (OZID), 1984.
ELLIS J.A. (2001): The immunology of the bovine respiratory disease complex. Vet Clin North Am Food Anim Pract 17; 535-537.
FULTON R.W. (2009): Bovine respiratory disease research (1983-2009). Animal Health Research Reviews 10(2); 131-139. doi:10.1017/S146625230999017X.
HODGSON P.D., AICH A., MANUJA A., HOKAMP H., ROCHE F.M., BRINKMAN F.S.I., POTTER A., BABIUK L.A., GRIEBEL P.J. (2005): Effect of stress on viral-bacterial synergy in bovine respiratory disease; novel mechanisms to regulate inflammation. Comp. Funct. Genom. 6; 244-250.
IRSIK M., LANGEMEIER M., SCHROEDER T., SPIRE M. AND RODER J.D. (2006): Estimating the effects of animal health on the performance of feedlot cattle. Bovine Practitioner 40; 65-74.
KURĆUBIĆ V., PETROVIĆ T., ĐOKOVIĆ R., ILIĆ Z., PETROVIĆ M.D. (2011): Antibody response of beef calves to experimental monovalent and multivalent inactivated bovine viral diarrhoea virus vaccines as measured by indirect ELISA method. Biotechnology in Animal Husbandry 27, (3), book 2: 901-911.
LAZIĆ S., PETROVIĆ T., BUGARSKI D., KENDRIŠIĆ N. (2009): Complex of respiratory diseases in cattle from the aspect of parainfluenca-3 virus. Biotechnology in Anim Husb 25 (5-6): 703-711.
LONERAGAN G.H., THOMSON D.U., MONTGOMERY D.L., MASON G.L., LARSON R.L. (2005): Prevalence, outcome, and health consequences associated with persistent infection with bovine viral diarrhea virus in feedlot cattle. J Am Vet Med, Assoc, 226: 595-601.
OIE (2008): Determining the presence of BVD virus genome (RT- PCR) and determining the presence of IBR/IPV virus genome (Real Time PCR). Manual of standards for diagnostic test and vaccines, Chapter 2.4.8 and 2.4.13 (2008); 6th Edition, Office International des epizooties, World organisation for animal health, OIE, Paris.
PARDON B., DE BLEECKER K., DEWULF J., CALLENS J., BOYEN F., CATRY B., DEPREZ P. (2011): Prevalence of respiratory pathogens in diseased, non-vaccinated, routinely medicated veal calves. Vet. Rec., 169; 278.

SNOWDER G.D., VAN VLECK L.D., CUNDIFF L.V., BENNETT G.L. (2006): Bovine respiratory disease in feedlot cattle: environmental, genetic, and economic factors. J. Anim. Sci. 84; 1999-2008.
TAYLOR J.D., FULTON R.W., LEHENBAUER T.W., STEP D.L., CONFER A.W. (2010): The epidemiology of bovine respiratory disease: What is the evidence for predisposing factors? Can. Vet. J. 51:1095-1102.
THONUR L., MALEY M., GILRAY J., CROOK T., LAMING E., TURNBULL D., NATH M. AND WILLOUGHBY K. (2012): One-step multiplex real time RT-PCR for the detection of bovine respiratory syncytial virus, bovine herpesvirus 1 and bovine parainfluenza virus 3. BMC Vet Res 8; 37. http://www. biomedcentral .com/1746-6148/8/37.

Received 17 August 2012; accepted for publication 20 December 2012

Biotechnology in Animal Husbandry 29 (1), p 45-51 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.083.312'62
DOI: 10.2298/BAH1301045K

INFLUENCE OF MICROCLIMATIC CONDITIONS ON THE DAILY PRODUCTION OF DAIRY COWS

D. Kučević[1], M. Plavšić[1], S. Trivunović[1], M. Radinović[1], V. Bogdanović[2]

[1]Faculty of Agriculture, Department of Animal Science, Novi Sad, 21000
[2]Faculty of Agriculture, Zootechnical Institute, Belgrade – Zemun, 11080
Corresponding author:
Denis Kučević; e-mail: denis.kucevic@stocarstvo.edu.rs
Original scientific paper

Abstract: The aim of this paper was to determine the microclimatic conditions (ambient temperature and relative humidity) in dairy farm, as well as to evaluate the effect and significance of temperature – humidity index (THI) values on the daily milk yield. The observation of microclimatic parameters was conducted in the period from 9.03.2012 to 6.05.2012. The study included 136 Holstein Friesian cows. The estimation of the effect of THI on daily production of dairy cows was defined by applying fixed-effect statistical model. Average ambient temperature during measuring months amounted to 15.6°C (ranging from 7.2° C to 24.6°C), while the average value of relative humidity was 56.33% (ranging from 40.30% to 81.80%). During the study, the mean value of THI was 58.93 (ranging from 47.08 to 70.13) and didn't exceed the critical comfort level of 72. All tested fixed-factors were statistically affected the daily milk yield ($p < 0.01$). For each unit of increase in the value of the THI, the amount of milk decreased by 0.05344 kg. This confirmed the importance of regular recording of THI values and microclimatic conditions as a unique indicator of thermal stress in dairy farm.

Key words: temperature – humidity index, dairy cows, milk yield, microclimatic parameters, heat stress

Introduction

Heat stress can have a very negative impact on milk production, reproduction and general health of cows *(Kadzere et al., 2002; West, 2003; Hansen, 2007).* In regard to heat stress, the most important factors are classified as ambient temperature and relative humidity *(Ravagnolo and Misztral, 2000; Bouraoui et al., 2002; Correa - Calderon et al., 2004).* Highly yielding dairy cattle in lactation show the most sensitivity to heat stress *(Cincović, 2010).* Influence of negative climatic factors may cause a decrease in milk production of lactating cows from 3% to 10% *(Hristov et al., 2007).* Acording to West *(2003)*, when the

ambiental temperature is 35°C, milk production decreases by 33%, and when the temperature is 40°C, milk production reduces to 50%. Thermoregulatory capabilities of cattle mostly depend on the ambient relative humidity level and temperature. Based on that, a unique indicator for environmental thermal stress was created, the temperature humidity index (THI) *(Mc Dowel et al., 1976)*.

THI is the most common and most accurate mean of temperature stress assessment *(Akyuz et al., 2010)* and will be used to determine the influence of heat stress on productivity of dairy cows. Milk production is affected by heat stress when THI values are higher than 72, which corresponds to 22°C at 100 % humidity, 25 °C at 50 % humidity, or 28 °C at 20 % humidity (*Du Preez et al., 1990a).*

Materials and Methods

The research was conducted from March 9th 2012 to May 6th 2012, on a dairy farm in Čantavir, Serbia. Cows were reared in a free system, capacity of 160 cattle in a single stable. The research included 136 Holstein cows. The facility used for the housing was divided into 5 departments with cow cubicles. The cows were grouped according to the lactation stadium. The horizontal ventilation was provided in the barn, whereas on the sides, curtains were added for additional micro climate regulation.

The amount of milk produced per cow, was measured by automated devices in the milking parlor. Temperature and humidity were measured every hour, during the experiment. Measurements were taken with three “data loggers” (Humidity and Temperature test 174H logger). The equipment was positioned in level with the cows withers, attached to the columns in each facility. The daily THI values were calculated using the equation by *Kibler (1964):*

THI = 1,8 Ta-(1-RH)(Ta-14,3)+32
where:
THI – temperature humidity index
Ta – temperature detected in stable
RH – relative humidity

Data was analysed using the software Statistics 10 *(stat. Soft. Inc. 2012)*. General variability of observed traits was analysed using the descriptive statistical analysis and the connection between the milk production and THI by the model of linear regression. Different sources of variability on daily milk yield were defined by applying the following statistical model:

$Y_{ijk}=\mu+L_i+S_j+b_1 (x_1 - \bar{x}_1)+ b_2(x_2 - \bar{x}_2)+ b_3 (x_3 - \bar{x}_3)+e_{ijk}$

Y_{ijk} – phenotypic value of observed traits

μ - population average
L_i – fixed effect of the parity
S_j – fixed effect of the calving season
$b_1(x_1 - \bar{x}_1)$ - linear regression effect of the age by calving
$b_2(x_2 - \bar{x}_2)$ - linear regression effect of the THI index
$b_3(x_3 - \bar{x}_3)$ - linear regression effect of the stage of lactation
e_{ijk} - other uncontrollable effects (random error)

Results and Discussion

Variations in the ambient temperature (Ta, ^{0}C), relative humidity (RH, %), and the THI in the stable recorded during the measuring months are reported in Table 1.

Table 1. Average values of microclimate conditions, THI and milk yield during measuring months[1]

Parameters	n	X	SD	CV	Se	min	max
Average milk yield	7746	24.62	7.48	30.40	0.09	1.60	49.50
Ta, ^{0}C	4173	15.63	4.42	28.26	0.58	7.20	24.60
RH, %	4173	56.33	11.45	20.33	1.49	40.30	81.80
THI	4173	58.93	5.75	9.76	0.75	47.08	70.13

[1]Ta - ambient temperature (°C); RH - relative humidity (%); THI - temperature-humidity index

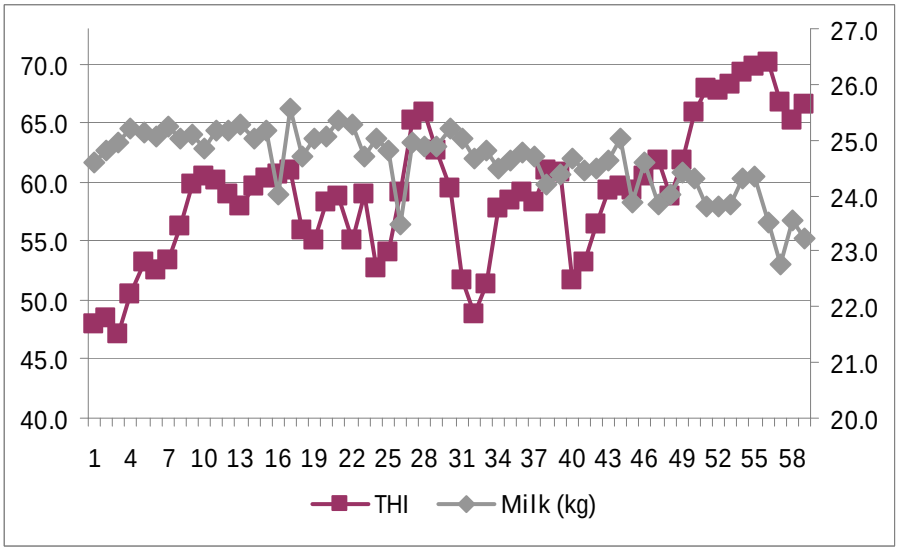


Figure1. Average temperature-humidity index and daily milk yield per cow during the period of observation

According to Table 1, average ambient temperature during measuring months amounted to 15.6°C (ranging from 7.2°C to 24.6°C), while the average value of relative humidity was 56.33% (ranging from 40.30% to 81.80%). During the study the mean value of THI was 58.93 (ranging from 47.08 to 70.13) and didn't exceed the critical comfort level of 72 (Figure 1). Optimal ambient temperature for dairy cows is 10°C to 15°C and comfort zone range from 5°C to 21°C *(Čobić and Antov, 1996). Kic and Brož (1995)* noticed that the optimal value of relative humidity ranges from 50% to 70% for lactating cows.

By temperatures higher than 26°C, cows reach a point where they are not able to cool themselves adequately, respectively to maintain constant body temperature and they enter the stage of temperature stress *(Kadzere et al., 2002).* The same authors find that THI values of 70 and less are comfortable, from 75 to 78 stressful, and values above 78 cause extreme danger, preventing the cows to maintain their normal body temperature.

Bouraoui et al. (2002) reported that if the THI value is between 35 and 72, the conditions for temperature stress occurrence are not met, and there are no conditions for the reduction of milk yield. *Akyuz et al. (2010)* noticed that the mild stress is experienced just when the value passes critical 72, moderate stress at 79 and at the end the dangerous level with values higher than 89.

Effects of THI and other observed factors (age by calving, stage of lactation, parity and calving season) on daily milk yield are shown in table 2.

Table 2. Effect of observed factors on daily milk yield[1]

Source of variability	d.f.	MS	F	p
Age at calving	9	2543.6	89.998	0.000000**
THI	1	746.6	26.417	0.000000**
Stage of lactation	1	182096.2	6443.043	0.000000**
Parity	1	2597.1	91.893	0.000000**
Calving season	1	1508.4	53.373	0.000000**

[1]d.f.= degrees of freedom; MS= mean square; F= f- value;
NS=P>0.05; *=P<0.05; **=P<0.01; ***=P<0.001;

The effects of the THI, age at calving, stage of lactation, parity and calving season were very highly significant (p<0.0001) for daily milk yield.

Coefficient of linear regression of the THI index on milk yield is shown in table 3.

Table 3. Value of the coefficient of linear regression[1]

	b	Std. error b	t- value	P - value
a	27.75637	0.871253	31.85797	0.000000**
THI	-0.05344	0.014642	-3.65001	0.000264**

Legend:1 b= coefficient of linear regression; a= intercept on the y – axis

Coefficient of linear regression of the THI index on milk yield was negative – 0.05344, which means that for each THI unit increase, milk yield decreases by 0.05344 kg.

Effect of THI on daily milk yield was also observed in other studies. *Cincović and Belić (2009)* have reported that, when THI reaches 72, a daily milk yield per cow decline by 0.2 kg. *West (2003)* stated that the daily milk yield per cow of Holstein breed decrease in average by 0.88 kg, per each unit of increase in THI. According to *Gantner et al. (2011)* the highest amount of daily loss (>0.9 kg/day) was determined in heifers.

Study of *Zimbelman et al. (2009)* has shown that the daily milk yield decreased around 2.2 kg/day by THI values from 65 to 73. *Bouraoui et al. (2002)* showed that when the THI index increases from 68 to 78, the decline of milk yield production totals 4kg, and for each THI unit increase, above 69, daily milk yield per cow reduces for another 0.41 kg. *Ravagnolo* (2000) determined that milk yield declined by 0.2 kg per unit increase in THI when THI exceeded 72. *Falta et al., (2008)* have also found that for THI values above 72, a milk yield decline of 4 kg occurs.

Conclusion

Based on the research of microclimate conditions (ambient temperature and relative humidity) as well as the effect of temperature-humidity index values on the daily production of dairy cattle, it could be emphasized that there were no conditions for the occurrence of heat stress during the experiment period because THI didn't exceed the critical comfort level of 72. The effects of the THI and other observed factors were very highly significant ($p<0.0001$) for daily milk yield. Amount of decrease of daily milk yield was not as high as in the results of other the authors, probably because the experiment was conducted in the spring when the critical limit of 72 is rarely exceeded. It has confirmed the importance of regular recording and monitoring of THI values and microclimatic conditions as a unique indicators of thermal stress on dairy farm, especially during the summer months when ambient temperature is around 40°C. Except monitoring of THI values, it is necessary to regulate the dairy management with the aim to minimize the effects of heat stress.

Uticaj mikroklimatskih uslova na dnevnu proizvodnju mleka krava

D. Kučević, M. Plavšić, S. Trivunović, M. Radinović, V. Bogdanović

Rezime

Cilj ovog rada je bio da se utvrde mikroklimatski uslovi (ambijentalna temperatura i relativna vlažnosti vazduha) i ispita uticaj različitih vrednosti temperaturno – humidnog indeksa (THI) na dnevnu proizvodnju mleka muznih krava. Period posmatranja mikroklimatskih parametara je sproveden u vremenu od 9.03.2012 do 6.05.2012. Ispitivanje je obuhvatilo 136 grla holštajn frizijske rase krava. Za procenu uticaja THI na dnevnu proizvodnju mleka korišten je statistički model sa uticajima fiksnih faktora. Prosečna temperatura ambijenta u toku ogleda je iznosila 15,6°C (kretala se od 7,2° C do 24,6°C) dok je prosečna relativna vlažnost vazduha iznosila 56,33% (kretala se od 40,30% to 81,80%). Za vreme istraživanja prosečna vrednost THI je iznosila 58,93 (kretala se od 47,08 do 70,13) i nije prelazila kritičan nivo komfora od 72. Svi ispitivani fiksni faktori su statistički značajno uticali na prinos mleka ($p<0,01$). Za svaku jedinicu porasta vrednosti THI, količina mleka se smanjivala za 0,05344 kg. Potvrđena je važnost redovnog praćenja THI i mikroklimatskih uslova kao jedinstvenog pokazatelja termalne stresogenosti sredine u kojoj borave krave muzare.

References

AKYUZ, A., BOYACI, S., CAYLI, A. (2010): Determination of critical period for dairy cows using temperature humidity index, Journal of Animal and Veterinary Advances, 9 (13), 1824 – 1827.

BOURAOUI R., LAHMAR M., MAJDOUB M., DJEMALI, M., BELYEA, R. (2002): The relationship of temperature – humidity index with milk production of dairy cows in a Mediterranean climate, Anim. Res. 51, 479 – 491.

CINCOVIĆ M. R. (2010): Toplotni stres krava – fiziologija i patofiziologija, Monografija, zadužbina Andrejević, Beograd,

CINCOVIĆ, M. R., BELIĆ B. (2009): Uticaj termalnog stresa krava na količinu i kvalitet proizvedenog mleka, Veterinarski žurnal Republike Srpske, vol. IX, broj 1, 53 – 56.

ČOBIĆ T., ANTOV G. (1996): Govedarstvo - prozvodnja mleka, S print, Novi Sad.

CORREA – CALDERON, A., ARMSTRONG D., RAY, D., DENISE S., ENNS M., HOWISON C. (2004): Thermoregulatory response of Holstein and Brown

Swiss heat – stressed dairy cows to two different cooling systems, Int. J. Biometeorol. 48, 142 – 148.
DU PREEZ J.H., GIESECKE W.H., HATTINGH P.J. (1990a): Heat stress in dairy cattle and other livestock under Southern African conditions. I. Temperature-humidity index mean values during the four main seasons. Onderstepoort J. Vet. Res. 57, 77-86.
FALTA D., WALTEROVA L., SKYPALA M., GHLADEK G. (2008): Effect of stable microclimate on milk production of Holstein cows on the 2nd and 3rd lactation, AWETH, vol 4. issue 2, 104-110.
GANTNER V., MIJIĆ P., KREŠIMIR KUTEROVAC, K., DRAGO, D., GANTNER R.: (2011) Temperature-humidity index values and their significance on the daily production of dairy cattle. Mljekarstvo 61 (1), 56-63.
HANSEN P. J. (2007): Exploitation of genetic and physiological determinants of embrionic resistance to elevated temperature to improve embryonic survival in dairy cattle during heat stress, Theriogenology, 68, S242 – S249.
HRISTOV S., STANKOVIĆ B., JOKSIMOVIĆ – TODOROVIĆ M., BOJKOVSKI J., DAVIDOVIĆ V. (2007): Uticaj toplotnog stresa na proizvodnju mlečnih krava, Zbornik naučnih radova, vol. 13, br. 3 – 4, 47 – 54.
KADZERE C. T., MURPHY M. R., SILANIKOVE N., MALTZ E. (2002): Heat stress in lactating dairy cows: a review, Livestock Production Science 77, 59-91.
KIBLER H. H. (1964): Environmental physiology and shelter engineering, LXVII, Thermal effect of various temperature – humidity combinations on Holstein cattle as measured by eight physiological response, Res. Bull. Missouri. Agric. Exp. Station., 862.
KIC P. A BROŽ V. (1995): Tvorba stajoveho prostředi, 1. vyd. Praha: Institut vychovy a vzdělavani Ministerstva zemědělstvi Českе republiky, 47 s. ISBN 80-7105-106-3.
MCDOWEL R. E., HOOVEN N. W., CAMOENS J. K. (1976): Effect of climate on performance of Holsteins in first lactation, J. Dairy Sci., 59, 965 – 973.
RAVAGNOLO O., MISZTRAL I. (2000): Genetic component of heat stress in dairy cattle, parameter estimation, J. Dairy Sci. 83, 2126 – 2130.
WEST, J. W. (2003): Effect of heat stress on production in dairy cattle. Journal of Dairy Science, 86: 2131-2144.
ZIMBELMAN R. B., RHOADS R. P., RHOADS M. L., DUFF G. C., BAUMGARD L. H., COLLIER R. J. (2009): A Re-evaluation of the impact of temperature humidity index (THI) and black globe humidity index (BGHI) on milk production in high producing dairy cows, Proc. 24th Ann. SW Nutr. Mgmt. Conf., 158 – 158.

Received 20 February 2013; accepted for publication 24 March 2013

Biotechnology in Animal Husbandry 29 (1), p 53-64 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC619:616.24'62
DOI: 10.2298/BAH1301053S

EVALUATION OF REVERSE TRANSCRIPTION-PCR PROTOCOLS BASED ON THE FUSION GENE FOR DIAGNOSIS OF BOVINE RESPIRATORY SYNCYTIAL VIRUS INFECTIONS

A. Selim [1], W. Gaede [2]

[1]Faculty of Veterinary Medicine, Benha University, P.O. Box 13736, Mostohor, Toukh, Egypt
[2]State Institute for Consumer Protection of Saxony-Anhalt, Dept. of Veterinary Medicine, P.O. Box D-39576, Haferbreiter Weg Str.132-135, Stendal, Germany
Corresponding author: abdelfattah.selim@fvtm.bu.edu.eg
Original scientific paper

Abstract: Bovine respiratory syncytial virus (BRSV) is a pneumovirus in the family paramyxoviridae, is an important cause of acute respiratory disease in postweaning calves and feedlot cattle. The real-time reverse transcriptase PCR protocols were developed to detect BRSV infection in infected animals. The sensitivity of RT-PCR protocols based on fusion gene were evaluated using different Mastermixes such as Qiagen One Step RT-PCR (Qiagen) for conventional RT-PCR, Superscrip probe (Invitrogen) and QuentiTec probe (Qiagen) for real-time RT-PCR with and without internal control. The detection limit of different RT-PCR protocols using serial dilutions from BRSV plasmid and based on different probes was 10 RNA copies/ml. Furthermore, the specificity of real-time RT-PCR was evaluated using different bacterial and viral strains which can be isolated from respiratory infected animals. In another side, the real-time RT-PCR in combination with ß-actin and conventional RT-PCR showed detectable Ct-values only with BRSV strain.

Keywords*:* BRSV, real-time RT-PCR, conventional PCR, internal control.

Introduction

Bovine respiratory syncytial virus (BRSV) is a fragile RNA, a single-stranded negative-sense RNA virus which belongs to the pneumovirus genus, a member of the Paramyxoviridae family. Its genome has approximately 15140 nucleotides and encodes 10 different mRNA molecules (*Buchholz et al., 1999; Grubbs et al., 2001*)

These viruses may cause respiratory tract infection and disease but often predispose the cattle to the bacterial pathogens. Additional viruses associated with

bovine respiratory disease. BRSV appears to be an important virus in the bovine respiratory disease complex because of its frequency of occurrence, predilection for the lower respiratory tract, and its ability to predispose the respiratory tract to secondary bacterial infection (*Ames, 1993*).

The virus is transmitted when an animal that is infected sheds the virus in secretions such as nasal discharge and a common example of this would be nose to nose contact (*Knight et al., 2001*). BRSV is transmitted horizontally by direct contact with respiratory secretions (aerosol infection). Infection is facilitated by crowding during the milking process and when animals are housed during the winter months. Newly acquired calves should be isolated and monitored for the presence of infection to prevent contamination of uninfected herds. BRSV infection causes severe respiratory signs in young cattle and frequently leads to the death of the infected animal (*Baker et al., 1997*).

The isolation of BRSV from clinically affected animals using conventional cell culture is challenging because of fragility of BRSV even in optimally stored samples. Thus, virus isolation attempts are often unsuccessful (*Kimman et al., 1986*). Therefore, the diagnosis of BRSV infection is more commonly performed through the detection of specific antibodies by sero-diagnostic methods such as complement fixation test and ELISA and indirect immunofluorescence test (IIF) as described by (*Westenbrink et al., 1987*).

IIF technique uses only a small portion of the entire organ, the infected area can be missed and requires special equipment (*Valarcher et al., 1999*). In contrast, diagnostic methods such as ELISA, virus isolation or real-time reverse transcriptase polymerase chain reaction (real-time RT-PCR) that uses organ homogenates extends the analysis to larger parts of the sample.

The BRSV genome encodes three glycoproteins: Nucleoprotein protein gene (N), the large attachment protein (G) and the fusion protein (F). The G and F proteins mediate binding of virus to cells and F is also responsible for fusion of viral and cell membranes. The fusion (F) protein coding region is less variable and therefore a more suitable target for the design of diagnostic tests (*Eleraky et al., 2003)*.

Classical methods of diagnosis based on BRSV antigen detection or virus isolation from lung samples and nasal swab demonstrated poor sensitivity due to low viral titers shedding (*Kimman et al., 1989a*). Consequently, several authors were developed different RT-PCR assays based on nucleoprotein gene (*Boxus et al., 2005),* against the fusion gene (*Hakhverdyan et al., 2005; Larsen et al., 1999*) and nested RT-PCR directed against the nucleoprotein gene (*Valarcher et al., 1999).*

In present study, a real-time RT-PCR assay with and without ß-actin internal control which targeting fusion gene was described, its sensitivity and specificity were evaluated using field infected samples. The sensitivity of this real-

time RT-PCR assay was compared with cell culture and conventional reverse transcriptase PCR.

Material and methods

Virus strains and culture

The bovine respiratory virus strain (RVB-017) was propagated on diploid cell line from primary calves lung cells containing 10% fetal calve serum, 1% glutamine and 1% nonessential amino acid. The virus also was maintained in MEM (Eagle's minimum essen medium containing 1 % gentamycin without fetal calve serum). The virus isolated from trachea of diseased cattle showed respiratory manifestation. The virus titer was determined as 50 % tissue culture infective dose ($TCID_{50}$), and inoculated the cell culture with 10^4 $TCID_{50}$ /ml. The cell culture was incubated at 37°C for 4 days. The virus was harvested then showing 90-100 % cytopathic effect. The infected cell was scrapped and the supernatant was clarified through centrifugation, liquated and stored at -70°C or RNA extracted with RNeasy mini kit for RT-PCR assay.

Preparation of BRSV plasmid

PCR products of primer set of RT-PCR that covering the entire sequence of the BRSV fusion protein (F) were cloned in the pCDNA3 plasmid (Invitrigen) leading to the pN constructs. pN plasmid was linearized with the restriction enzyme XbaI (Roche Diagnostics GmbH, Mannheim, Germany). N control RNAs was transcribed from the T7 promoter with T7/SP6 RNA transcription kit (Roche Diagnostics GmbH, Mannheim, Germany) as recommended by the manufacturer. A purification step was performed using the RNeasy mini Kit (Qiagen, Hilden, Germany) to remove non-incorporated nucleotides.

Extraction of viral RNA

BRSV-RNA was extracted using RNeasy mini Kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. 30 mg tissue or nasal swab transport medium or 600 µl cell pellets (5 x 10^6 cells) lysed in 600 µl RLT buffer and then homogenized. Ethanol is added to the lysate to provide ideal binding conditions. The lysate is then loaded onto the RNeasy silica membrane. RNA binds and all contaminants are efficiently washed away. Pure and concentrated RNA is eluted in water.

Conventional PCR

Conventional PCR was carried out in 25 µl reaction volume. PCR mixtures contained 0.75 µl working solution of both forward primer (BRSV S1, 20 pmol/µl) and reverse primer (BRSV S2, 20 pmol/µl) described by *(Oberst et al. 1993)*, 14,4

µl Nuclease-free water (Qiagen, Hilden, Germany), 5.0 µl PCR buffer (Qiagen® 5x), 1.0 µl dNTP (10 mM of each dATP, dCTP, dGTP,and dTTP; Qiagen), 0.1 µl RNase inhibitor and 1.0 µl RT-Mix (Qiagen, OneStep RT-PCR). Finally 2.0 µl RNA template were added. All primers were delivered by TIB MOLBIOL GmbH (Berlin, Germany) as can be seen Table 1.

The PCR reaction was performed on Thermocycler (MasterCycler, Eppendorf, Hamburg, Germany) as follow: a reverse-transcription reaction temperature at 50°C for 30 min and initial PCR activation of Taq-polymerase for 15 min at 95°C followed by 49 cycles of 30 sec at 94°C, 30 sec at 64°C, 1 min at 72°C and finally one extension cycle at 72°C for 10 min.

The PCR product was detected by electrophoresis through a 2 % gel stained with ethidium bromide and visualized under UV light.

Protocol of single real-time RT-PCR assay

The single real-time RT-PCR reaction was performed in 20 µl reaction volume. PCR mixtures contained 0.8 µl working solution of both primer BRSV-sdl-R and BRSV-sdl-R, 0.8 µl BRSV-probe (1pmol/µl), 10.0 µl QuantiTect probe RT-PCR Mastermix (Qiagen, Hilden, Germany) and 0.2 µl QuantiTect probe RT-Mix (Qiagen, Hilden, Germany). Finally 5.0 µl template was added. The primer concentration in working solutions was 10 pmol/µl. All primers were delivered by TIB MOLBIOL GmbH (Berlin, Germany) as can be seen in Table1.

The PCR reaction was performed in Stratagene thermocycle with the following programe: RNA is reverse-transcribed at 50°C for 30 min initial PCR activation of Taq-polymerase for 15 min at 95°C followed by 45 cycles of 30 sec at 94°C, 30 sec at 60°C, 30 sec at 60°C.

The single real-time RT-PCR was performed also using another MasterMix. PCR mixture was contained 3.5 RNase free water, 0.5 µl of each BRSV-sdl forward and reverse primers, 2.5 µl BRSV TaqMan probe, 0.5 µl RT-mix (Superscript III RT/ Platinum-Mix, Invitrogen), 12.5 µl PCR buffer. Finally 5.0 µl template was added. The primer concentration in working solutions was 10 pmol/µl. All primers were delivered by TIB MOLBIOL GmbH (Berlin, Germany), see Table1.

The PCR reaction was performed in Stratagene thermocycle with the following programe: RNA is reverse-transcribed at 50°C for 30 min initial PCR activation of Taq-polymerase for 2 min at 95°C followed by 55 cycles of 30 sec at 94°C, 30 sec at 58°C, 30 sec at 68°C and finally cooling at 37°C for 30 sec.

Protocol of Duplex real-time RT-PCR assay

The duplex real-time RT-PCR reaction was performed in 20 µl reaction volume. PCR mixtures contained 2 µl of BRSV mix-1 [200 µl mix prepared from 20 µl of BRSV-sdl forward and reverse primer 2.5 µl BRSV-FAM probe and 157.5 µl 0.1 x tris EDTA buffer (pH 8)], 2 µl ß-actin mix-2 [200 µl mix 2 prepared from

5 µl of ACT forward and reverse primer, 2.5 ACT-HEX probe, 187.5 µl 0.1 x tris EDTA buffer (pH 8)], 0.8 µl RNase free water, 10 µl QuentiTect Probe RT-PCR MasterMix and 0.2 µl QuantiTect Probe RT-Mix. Finally, 5.0 µl template was added. The primer concentration in working solutions was 100 pmol/µl. All primers were delivered by TIB MOLBIOL GmbH (Berlin, Germany) as can be seen in Table 1.

The PCR reaction was performed in Stratagene thermocycle with the following program: RNA is reverse-transcribed at 50°C for 30 min initial PCR activation of Taq-polymerase for 15 min at 95°C followed by 45 cycles of 30 sec at 94°C, 30 sec at 60°C, 30 sec at 60.

Table 1. Primers and probes for BRSV-detection

Application	Primer	Sequence	Acc-No	Position	Product Size (bp)	Reference
real-time RT-PCR	BRSV-sdl-F	5´-ACA CCC CCT GTT GGA AAC TAC A-3´	FJ543092	914-935	66	This study
	BRSV-sdl-F	5´-AAA AGA CAC AGA GCC TGC ATT GTC AC-3`		1038-1013		
	BRSV-TaqMan	Cy5-ACC ACC CAC GAT CTG TCC TAG TTA AGC A-BBQ		1009-982		
Conventional RT-PCR	BRSV-S1	5´- TTA CCA CAC CCC TCA GTA CA-3´	M58350	741-760	381	This study
	BRSV-S2	5´- CAT TGT GTC ACA GAA CAC TC-3´		1123-1104		
Internal control (ß-actin)	ACT-1005-F	5´- CAG CAC AAT GAA GAT CAA GAT CAT C-3´	DQ838049	966-990	129	This study
	ACT-1135-R	5´- CGG ACT CAT CGT ACT CCT GCT T- 3´		1096-1075		
	ACT-1081-TaqMan	HEX- TCG CTG TCC ACC TTC CAG CAG ATG T- BHQ1		1042-166		

Determination of analytical sensitivity

The sensitivity of conventional Qiagen one step RT-PCR and single real-time RT-PCR assay was evaluated using Superscript and QuantiTect MasterMix probe and duplex real-time RT-PCR with ß-actin was evaluated with serial dilution from BRSV control virus ranged between10^7 to10^1 RNA-copies/ml and also with ten-fold serial dilution of BRSV plasmid ranged between between10^7 to10 RNA-copies/ml.

Analytical specificity

The specificity of both PCR assays was evaluated by reference control of etiological cause of different diseases agents either viral or bacterial organism can cause respiratory manifestation and misdiagnosis with BRSV or cause secondary infection to BRSV infection. These agents such as bovine herpes virus1 and 4,

bovine viral diarrhea, bovine para-infulenza 3, Malignant catarrhal fever, bovine respiratory syncytial virus and bacterial causes such as *Brucella abortus, Chlamydian psittacii, Coxiella burnettii, Campylobacter fetus, E. coli, Leptospira spp., Listeria monocyotgenes, Mycoplasma bovis, Neopsora caninum and Mycobacterium avium subsp. paratuberculosis.*

Diagnostic sensitivity

We examined 87 naturally infected animals through collected nasal swabs from animals showed respiratory symptoms. These samples were extracted with RNeasy mini kit (Qiagen) and examined with conventional PCR in parallel to single and duplex real-time RT-PCR to determine the diagnostic ability of different examined PCR assays to field infection.

Assessment of PCR efficiency

Assessments of the amplification efficiency and the precision of the assay under optimized conditions were performed by serial dilutions of reference virus strain and BRSV-cloning plasmid. Standard curve construction was performed for real-time RT-PCR and the slopes were used for the calculation of amplification efficiency (E) by using the equation $E=10^{(-1/slope)} -10$

The robustness of the assay was investigated as follows. Duplicates of the serial dilutions of reference virus strain were inoculated on cell culture and analysed with conventional and real-time RT-PCR assay under optimized concentration of PCR reagents.

In addition, the influence of different MasterMix PCR reagents were investigated as Qiagen® One step RT-PCR kit (Qiagen) for conventional Rt-PCR, QuantiTect probe RT-PCR Master mix Kit (Qiagen) Superscript III RT/ Platinum –Mix kit (Invitrogen, California, USA) for real-time RT-PCR assay.

Results and Discussion

Analytical sensitivity

BRSV control virus

The results of analytical sensitivity based on serial dilution of virus control and type of RT-PCR assay were differed. The detection limit of single real-time RT-PCR protocol using QuentiTec (Qiagen) and superscript (Invitrogen) probe and duplex real-time RT-PCR using QuentiTec probe (Qiagen) based on serial dilution of virus PK 1/06 was 10^2 RNA copies but higher than the detection limit of conventional RT-PCR which was performed using QuentiTec one-step PCR probe (Qiagen), it was 10^3 RNA copies as can be seen in Table 2.

PCR efficiency of real-time RT-PCR was evaluated with QuantiTect probe and with Superscript probe based on serial dilution of virus control strain PK 1/06 and for duplex real-time RT-PCR with QuentiTec probe.

In principles, the correlation coefficients (r^2) of real time RT-PCR with the two probes and duplex real-time RT-PCR with QuentiTec probe assay were exceed 0.9866 and showed linear relationship between Ct-values and the corresponding serial dilution of BRSV virus.

Table 2. Analytical sensitivity of different PCR protocols based on serial dilution of BRSV control strain and different Mastermix probe.

PCR assay	RT-PCR	Real-time RT-PCR		Duplex real-time RT-PCR	
Mastermix Probe	Qiagen one-step PCR	QuantiTec (Qiagen)	Superscript (Invitrogen)	QuantiTec probe(Qiagen)	
Virus (PK 1/06)				Ct-Values	ß-actin
1.00E-00	positive	24.0	25.7	21.9	35.4
1.00E-01	positive	27.8	28.3	24.0	36.4
1.00E-02	positive	30.5	31.6	27.3	37.3
1.00E-03	positive	33.4	34.9	30.5	32.3
1.00E-04	negative	36.0	37.9	33.9	34.6
1.00E-05	negative	40.0	39.9	37.0	33.5
Regression coefficient	-	0.9957	0.9866	0.9866	

BRSV plasmid

The sensitivity of different RT-PCR protocols was evaluated base on serial dilution of BRSV plasmid.

Table 3. Analytical sensitivity of PCR protocols based on serial dilution of BRSV Plasmid and different Mastermix probe.

PCR assay	Conventional RT-PCR	Real-time RT-PCR		Duplex real-time RT-PCR	
Mastermix Probe	Qiagen One step	QuantiTec (Qiagen)	Superscript (Invitrogen)	QuantiTec probe(Qiagen)	
BRSV plasmid				Ct-Values	ß-actin
1.00E+08	positive	14.2	16.5	14.5	38.3
1.00E+07	positive	17.0	20.9	18.1	38.2
1.00E+06	positive	20.7	24.7	21.3	36.3
1.00E+05	positive	23.9	27.3	25.1	37.5
1.00E+04	positive	27.7	29.9	27.9	36.1
1.00E+03	positive	31.1	33.9	31.7	35.8
1.00E+02	positive	35.0	36.6	34.7	38.2
1.00E+01	positive	38.2	36.9	38.2	37.3
1.00E+00	positive	No Ct	No Ct	No Ct	37.2
Regression coefficient		0.9994	0.9963	0.9992	

The results revealed similar sensitivity between conventional RT-PCR and real-time RT-PCR either with QuentiTect (Qiagen) or Superscript (Invitrogen) Probe and duplex real-time RT-PCR based on QuentiTec probe (Qiagen) that was about 10 RNA copies/PCR and results showed detectable ct-values of ß-actin with all dilutions as can be seen in Table 3.

There was linear relationship between threshold cycle and the concentration of BRSV plasmid. Regression coefficient (r^2) was calculated that was in case of real time RT-PCR assay 0.999 and 0.996 with QuentiTec and Superscript probe respectively and 0.999 in duplex real-time RT-PCR assay as can be seen in Figure 1 and Table 4. Consequently, the PCR efficiency of real-time RT-PCR with serial dilution of BRSV plasmid was evaluated 94.54% and 109.60, 98.99% in QuentiTec and Superscript probe of single real-time RT-PCR assay and duplex real-time RT-PCR assay respectively as shown in Table 4.

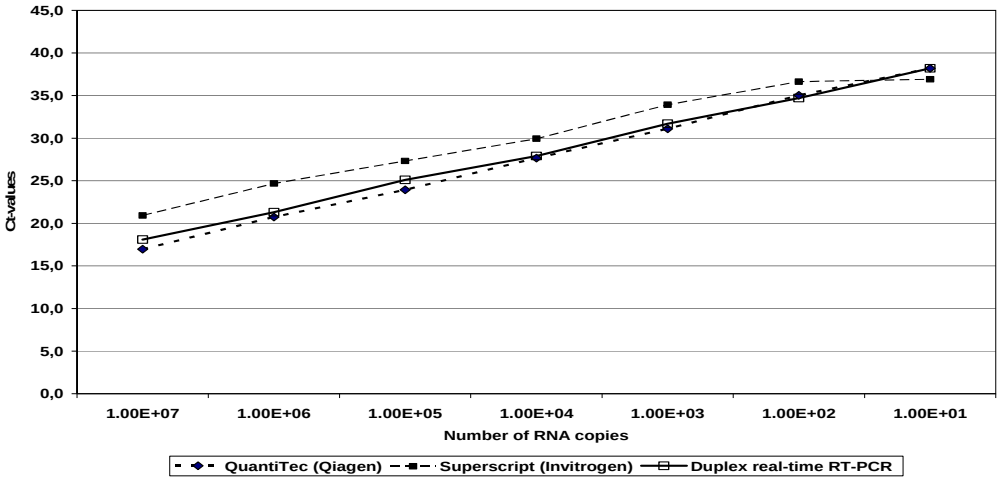


Figure 1. Relationship between Ct-values and the log-concentration of BRSV plasmid.

Table 4. Data of determination of analytical PCR sensitivity according to BRSV plasmid.

Method	MasterMix Probe	Dilution of BRSV plasmid (according to table 3)		
Parameter		r^2	PCR-efficiency	Detection limit
Real-time RT-PCR	Superscript	0.996	109.60%	10
	QuantiTec	0.999	90.54%	10
Duplex real-time RT-PCR	QuantiTec	0.999	98.99%	10
Conventional RT-PCR	Qiagen one step			10

Analytical specificity

The spectrum of detection of conventional RT-PCR and real-time RT-PCR was varied by amplifying RNA and DNA extracted from different bacteria and viruses associated with respiratory disease and can cause cross reaction with BRSV. The conventional RT-PCR showed defined PCR product only with BRSV and has never any detectable product with other microbial species. In another hand, the single real-time RT-PCR revealed detectable Ct with BRSV and was negative with other microbial species but showed weak positive signal with 38.5 Ct values with *Mycoplasma bovis* strain which extracted from milk sample. In repetition with the same *Mb.bovis* strain and the result revealed negative result which mean presence of cross contamination in first assay with BRSV. Furthermore, the duplex real-time RT-PCR showed only detectable Ct with BRSV strain and hasn't got any cross reaction with other tested species and combined with detectable Ct-values of ß-actin internal control.

Diagnostic sensitivity

After examination of 87 field samples of respiratory disease animals, the conventional RT-PCR showed 70 positive samples from 87 totals. In contrast, the single real-time RT-PCR showed 73 positive compared to 69 positive in case of duplex real-time PCR which has got detectable Ct-values of ß-actin internal control in all samples even with false negative result.

BRSV is one of the most important respiratory viruses due to its ability to cause infection to respiratory tract and its ability to predispose the respiratory tract to secondary bacterial infection (*Ames et al., 1993*). Presumptive diagnosis of BRSV can be determine depending on clinical feature of disease in infected animal or epidemiological occurrence of disease in herd. Lack of definite BRSV diagnosis because of inadequate viral isolation using cell culture and due to fragile nature of the virus. Consequently, direct detection of BRSV antibodies was performed using the serological test has become the standard method for BRSV diagnosis. In the present, several authors have used PCR as more sensitive test for diagnosis such as nested PCR with two round of amplification to detect the virus in clinical samples (*Belak and Thoren, 2001*) but the nested PCR have several disadvantage such as it is a bit complex due to it needs two round of amplification and a gel elctrophoresis which lead to high risk of contaminations.

These lead to important need to developed new simple and reliable diagnostic method for BRSV detection in clinical samples as quantitative RT-PCR which can be performed in a single-step and in closed-tube with fluorescence TaqMan detection probe. Several real-time PCR assays were developed according to nucleoprotein gene of BRSV (*Valacher et al., 1999; Boxus et al., 2005*). In another side, there is other studies were developed according to Fusion gene due to its stabile nature and less variable as (*Hakhverdyan et al., 2005; Vilcek et al., 1994; Larsen et al., 1999*).

In order to obtain highly sensitive PCR assay, we developed a real-time RT-PCR assay and the sensitivity of this assay was performed includes ß-actin internal control and without internal control using varied BRSV sources such as BRSV control virus isolated from clinical samples and cloning plasmid virus. The results of real-time RT-PCR assays was also compared with conventional PCR. The sensitivity analysis with BRSV reference strain revealed that the detection level of both conventional and real-time RT-PCR assays was similar at 10 RNA-copies/ml. As far as, the detection limit of real-time RT-PCR assay using different MasterMix probe according to BRSV control strain was higher sensitive than conventional RT-PCR and similar to duplex real-time RT-PCR with ß-actin table 2. Furthermore, the detection level of the three PCR assays was similar in case of using serial dilution that prepared from virus plasmid, it was 10 RNA-copies/ml.

Our data corresponding to other studies which have about similar detection range such as *Hakhverdyan et al.* (2005) developed fluorogenic reverse transcription PCR depend on F-gene and its detection level was 10 RNA-copies/ml, in nested PCR depend on F and G gene was 0.1 $TCID_{50}$ (*Vilcek at al., 1994*). Furthermore our sensitivity detection level of both conventional and real-time RT-PCR was higher than another assay as detection level of conventional RT-PCR was 10^5 RNA copies/ml (*Larsen et al., 1999*) and 10^3 RNA copies/ml of real-time RT-PCR assay depend on nucleoprotein gene (*Boxus et al., 2005*).

The specificity of our developed PCR assay was evaluated with different viral and bacterial species which can cause respiratory disease in animal and make misdiagnosis to BRSV or cause secondary infection to BRSV infection. Our result of conventional RT-PCR and duplex real-time RT-PCR assays was revealed detectable Ct only with BRSV control virus which mean highly specific test to BRSV diagnosis. In contrast real-time RT-PCR was revealed weak Ct-value with Mycoplasma bovis strain which isolated from milk sample but in repetition this sample back to be negative which mean presence of contamination.

In our practical use to the three PCR assays for detection of BRSV in clinical samples of natural infected animal revealed that the real-time RT-PCR assay is most reliable and rapid test for BRSV diagnosis. The result of real-time RT-PCR assay showed 73 positive samples against 70 and 69 positive sample from 87 totals in conventional and duplex real-time RT-PCR, respectively. This result showed bit of competition between the primer of BRSV and ß-actin that lead to low sensitive assay and also conventional RT-PCR has still risk of electrophoresis contamination and handling complex.

Conclusion

The result revealed that real-time RT-PCR is most a rapid, sensitive and specific assay for detection of BRSV in clinical samples and easily applied in routine diagnosis.

Ocena protokola reversne transkripcije PCR na bazi fuzionog gena za dijagnozu infekcije respiratornim sincicijalnim *virusom kod goveda*

A. Selim, W. Gaede

Rezime

Respiratorni sincicijalni *virus kod goveda* (BRSV), pneumovirus iz porodice *paramyxoviridae* je važan uzrok akutnog respiratornog oboljenja teladi nakon odbijanja i goveda u tovilištu. PCR protokoli reverzne transkriptaze u realnom vremenu su razvijeni da otkriju BRSV infekciju u zaraženim životinjama. Osetljivost RT-PCR protokola na osnovu spajanja gena su ocenjeni korišćenjem različitih Mastermixes kao što su Qiagen One Step RT-PCR (Qiagen) za konvencionalnu RT-PCR, Superscrip probe (Invitrogen) i QuentiTec probe (Qiagen) RT-PCR u realnom vremenu, sa i bez unutrašnje kontrole. Granica detekcije različitih RT-PCR protokola koji koriste serijski rastvore iz BRSV plazmida i zasnovane na različitim probama, bilo je 10 RNK kopija / ml. Osim toga, specifičnost RT-PCR u realnom vremenu je ocenjen korišćenjem različitih bakterijskih i virusnih sojeva koji mogu biti izolovani kod životinja sa respiratornim zaraznim oboljenjima. S druge strane, RT-PCR u realnom vremenu u kombinaciji sa ß-actin i konvencionalnom RT-PCR, pokazala je uočljive CT-vrednosti samo sa BRSV sojem.

References

AMES T.R. (1993): The epidemiology of BRSV infection. Veterinary Medicine 88, 881-885.

BAKER JC, ELLIS JA, CLARK EG (1997): Bovine respiratory syncytial virus. Veterinary Clinics North American food Animal Practice, 13, 425-454.

BELAK S., THOREN P. (2001): Molecular diagnosis of animal disease: some experiences over the past decade. Expert Review Molecular Diagnostics, 1, 434-443.

BOXUS M., LETELLIER C., KERKHOFS P. (2005): Real-time RT-PCR for the detection and quantification of bovine respiratory syncytial virus. Journal of Virological Methods, 125, 125-130.

BUCHHOLZ U.J., FINKE S., CONZELMANN K.K. (1999): Generation of bovine respiratory syncytial virus (BRSV) from cDNA: BRSV NS2 is not essential for virus replication in tissue culture, and the human RSV leader region acts as a functional BRSV genome promoter. Journal of Virology, 73, 251-259.

ELERAKY N.Z., KANIA S., POTGEITER L.N.D. (2001): The ovine respiratory syncytial virus F gene sequence and its diagnostic application. Journal of Veterinary Diagnostic Investigation, 13, 455-461.
GRUBBS S.T., KANIA S.A., POTGIETER L.N. (2001) Validation of synthetic peptide enzyme immunoassays in differentiating two subgroups of ruminant respiratory syncytial virus. Journal of Veterinary Diagnostic Investigation, 13, 123-127.
HAKHVERDYA M., HÄGGLUND S., LARSEN L.E., BELAK S. (2005): Evaluation of a single-tube fluorogenic Rt-PCR assay for detection of bovine respiratory syncytial virus in clinical samples. Journal of Virological Methods, 123, 195-202.
KIMMAN T.G., ZIMMER G.M., STRAVER P.J., DE LEEUW P.W. (1986): Diagnosis of bovine respiratory syncytial virus infections improved by virus detection in lung lavage samples. American Journal of Veterinary Research, 47, 143-147.
KIMMAN T.G., WESTENBRINK F., STRAVER P.J. (1989a): Priming for local and systemic antibody memory response to bovine respiratory syncytial virus: effect of amount of virus, virus replication, route of administration and material antibodies. Veterinary Immunology Immunopathology, 22, 145-160.
KNIGHT D.M., HOWLEY P.M., GRIFFIN D.E., LAMB R.A., MARTIN M.A., ROIZMAN B., STRAUS S.E. (2001): Fundamental virology. 4th ed. Lippincott Williams and Wilkins, Philadelphia, 395.
LARSEN L.E., TJORNEHOJ K., VIUFF B., JENSEN N.E., UTTENTHAL A. (1999): Diagnostic of enzootic pneumonia in Danish cattle: reverse transcription-polymerase chain reaction assay for detection of bovine respiratory syncytial virus in naturally and experimentally infected cattle. Journal of Veterinary Diagnostic Investigation, 11, 416-422.
TOUSSAINT J.F., SAILLEAU C., BREARD E., ZIENTARA S., DE CLERCQ K. (2007): Bluetongue virus detection by two real-time RT-qPCRs targeting two different genomic segments. Journal Virological Methods, 140, 115–123.
VALARCHER J.F., BOURHY H., GELFI J., SCHELCHER F. (1999): Evaluation of a nested reverse transcription-PCR assay based on the nucleoprotein gene for diagnosis of spontaneous and experimental bovine respiratory syncytial virus infections. Journal of Clinical Microbiology, 37, 1858-1862.
VILCEK S., ELVANDER M., BALLAGI-PORDANY A., BELAK, S. (1994): Development of nested PCR assays for detection of bovine respiratory syncytial virus in clinical samples. Journal of Clinical Microbiology, 32, 2225-2231.
WESTENBRINK F., KIMMAN T.G. (1987): Immunoglobulin M-specific enzyme-linked immunosorbent assay for serodiagnosis of bovine respiratory syncytial virus infections. American journal of Veterinary Research, 48, 1132—1137.

Received 20 February 2013; accepted for publication 24 March 2013

Biotechnology in Animal Husbandry 29 (1), p 65-74 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.06'636.38
DOI: 10.2298/BAH1301065Y

PRINCIPAL COMPONENT ANALYSIS OF THE CONFORMATION TRAITS OF YANKASA SHEEP

A. Yakubu

Department of Animal Science,
Faculty of Agriculture,
Nasarawa State University, Keffi, Shabu-Lafia Campus, P.M.B. 135, Lafia, Nigeria.
Corresponding author': abdul_mojeedy@yahoo.com
Original scientific paper

Abstract: This study was preformed to evaluate the biometric traits of 227 Yankasa sheep in northern Nigeria under a multivariate approach. The body measurements taken were: withers height, rump height, body length, heart girth, tail length, face length, shoulder width, head width, rump width, ear length, foreleg length, hind leg length and rump length. The animals were divided into two age groups: <15.5 and 15.5 – 28.3 months old, respectively. General linear model was used to study age group effect while principal component factor analysis was performed to define body shape upon the correlation matrix of the thirteen body measurements. Age group significantly ($P<0.05$) affected the morphological characters except ear length. Pearson's coefficients of correlation were positive and significant in both age groups. In <15.5 months old sheep, four principal components (factors) were extracted (ratio of variance = 89.27). The first factor accounted for 73.03% of the total variance and was interpreted as a measure of general size. The second factor which explained 7.61% of the generalized variance tended to describe flesh dimensions (shoulder width and rump width), while the third factor had its loadings for tail length and ear length. The fourth factor was influenced by head width. In 15.5–28.3 months old sheep, three factors (ratio of variance=75.21) were identified. These seven extracted factors could be considered in breeding programmes to improve body conformation of sheep since variation in meat traits was not associated with body height.

Key words: Yankasa sheep, body dimensions, age group, principal components, breeding

Introduction

Body composition and growth performance are important to assess the potential of development in animals. External body measurements of animals have

been extensively used to assess the growth of skeletal parts; and to describe the changes in animal conformation with age (*Ngere et al., 1984*). Morphometric characters are easy to monitor (*Herrera et al. 1996; Yakubu and Akinyemi, 2010*) and they facilitate the use of ethnological characterization and at the same time institute reliable racial discriminants.

Since conformation and some production traits are intercorrelated both genetically and phenotypically (*Brown et al., 1973; Shahin, 1996; Yakubu and Mohammed, 2012*), the analysis of zoometrical variables could be considered in selection programmes to acquire highly coordinated animal bodies. Factor analysis is a multivariate methodology that can be employed when characteristics are correlated, thereby describing objectively the underlying dimension of size and shape. It permits the elimination of redundancies from sets of interdependent variables, extract and identify covariant variable sets that are statistically unrelated (*Nugent and Notter, 1991; Shahin et al., 1995; Yakubu et al., 2011).*

Yankasa sheep are the third largest breed of sheep in Nigeria. However, there is general dearth of information on their body measurements using a multivariate approach. The present investigation therefore aimed at documenting changes in the morphological indices of Yankasa sheep across two age groups. It equally examined the interdependence among body measurements with a view to reducing the number of traits for genetic and breeding purposes using varimax rotated principal component factor analysis (*Posta et al*., 2007).

Materials and Methods

The study was carried out in Lafia, Nasarawa State, which falls within the guinea savannah zone of north central Nigeria. It is located between latitude 08° 35´N and longitude 08° 33´E

Experimental animals

Two hundred and twenty seven Yankasa rams reared through the extensive system of management were randomly measured. The animals were carefully observed to avoid measurements from crossbreds and unhealthy ones. The sheep were divided into two age groups namely < 15.5(n=92) and 15.5–28.3(n=135) months old. The age was determined using the number of permanent incisors as described by Wilson and Durkin (1984) who worked on indigenous sheep and goat breeds of semi- arid Africa. Animals were categorized as follows:

Number of permanent incisors	**Age (months)**
0 (milk teeth)	< 15.5
1-2 pairs (2-4)	15.5-28.3

Body parts measured

Thirteen metric traits were measured on each animal following standard procedure and anatomical reference points described by Yakubu *et al*. (2005). The body parts consisted of withers height (WH), distance between the most cranial palpable spinous process and the ground, rump height (RH), distance from the top of the pelvic girdle to the ground, body length (BL), measured from distance between the tip of scapula to tail drop, heart girth (HG), body circumference immediately behind the forelegs, face length (FAL), distance from between the horn site to the lower lip, foreleg length (FL), distance from the proximal extremity of the olecranon process to the mid-lateral point of the coronet. Hind leg length (HL), measured up to the mid-lateral point of the coronet, and rump width (RW), width between the hip bones (*Tuber coxae*), rump length (RL), measured from hips (*Tuber coxae*) to pins (*Tuber ischii*), shoulder width (SW), measured as a distance from left to right upper arm, and head width (HW), measured as the widest point of the head, tail length (TL), measured from tail drop to tip of the tail, and ear length (EL), the distance from the point of attachment to the tip of the ear. A graduated measuring stick was used for the height measurements, the length and circumference measurements were done using a flexible tape, while a special wooden calliper was used for the width measurements.

Statistical analysis

Data collected were subjected to analysis of variance (ANOVA) using the general linear model. Age group was the factor included in the model as a source of variation. Pearson's coefficients of correlation (r) among the various body measurements were estimated. From the correlation matrix, data were generated for the principal component factor analysis. This involved the use of the factor programme of SPSS (2001).

According to Johnson and *Wichern (2002)*, principal components are linear combination of the original variables and are estimated in such a way that the first principal component explains the largest percentage of the total phenotypic variance. The varimax criterion of the orthogonal rotation method was employed for the rotation of factor matrix. The choice of varimax rotation is informed by its ability to maximize sum of the variances of the squared loadings within each column of the loading matrix. This tends to produce some higher loadings and some loadings near zero which is one of the aspects of simple structure that enhance the interpretability of the factors. Considering a P-variate system, the principal component is given in expression as

$PC_i = a_{i1} X_1 + a_{i2} X_2 +$----------$a_{ij} X_j$
with
i = 1,2,----------, n principal components and

j = 1,2,----------, p original variables
where,
a_{ij} = the jth component of the coefficient vector of the linear transformation
X_j = the original variable

Results and Discussion

Morphological traits

The mean, standard deviation and coefficient of variation for each of the recorded linear body measurements of the two age groups are presented in Table 1. Generally, the linear body dimensions for 15.5 – 28.3 months old sheep were significantly higher ($p<0.05$) than of those <15.5 months old except ear length. The animals were found to be taller at the withers than at the rump, sloping gently backwards. The marked differences observed in the variables of the two age groups are not surprising since component parts of the animals are expected to increase differentially as the animal grows with age (*Salhab et al., 2001; Yakubu, 2003*). Higher variability was observed in the body parameters of less than 15 months old sheep compared to those of 15.5 – 28.3 months old. Tail length, ear length, rump width, heart girth, shoulder width and body length were more variable in the former (CV= 21.84, 20.41, 16.79, 14.57, 14.37 and 14.03 respectively) while in the latter, variability was highest in tail length followed by ear length, head width, shoulder width, heart girth and rump width (CV = 19.36, 17.19, 15.90, 11.20 and 10.60 respectively). The coefficient of variation values obtained for heart girth, shoulder width and rump width might reflect the sensitive response of these measurements to changes in the fitness (condition) of an animal.

Table 1: Means, standard deviations (SD) and coefficients of variation (CV) of the body measurements of Yankasa sheep

	<15.5 months Old (n=92)			15.5 -28.3 Months Old (n = 135)		
Traits (cm)	Mean	SD	CV	Mean	SD	CV
Withers height	60.48	7.92	13.10	67.86	5.42	7.99
Rump height	59.67	7.51	12.59	66.66	5.15	7.73
Body length	57.10	8.01	14.03	66.10	6.50	9.83
Heart girth	65.00	9.47	14.57	76.58	8.58	11.20
Tail length	25.59	5.59	21.84	28.72	5.56	19.36
Face length	20.22	2.44	12.07	23.01	2.20	9.56
Shoulder width	14.13	2.03	14.37	16.71	1.90	11.37
Head width	8.17	0.96	11.75	9.37	1.49	15.90
Rump width	12.51	2.10	16.79	15.75	1.67	10.60
Ear length	10.68	2.18	20.41	10.76	1.85	17.19
Foreleg length	41.61	4.04	9.71	44.40	3.51	7.91
Hind leg length	38.27	3.10	8.10	41.40	3.06	7.39
Rump length	18.95	2.47	13.03	21.55	2.06	9.56

Correlation analysis

The correlations were all positive and significant (P<0.05) as shown in Table 2. However, the magnitude of the correlations among the morphological characters was higher in <15.5 months old sheep (upper matrix) compared to their 15.5-28.3 months old counterparts (lower matrix). This is not astounding as it lends credence to the rapidity in the growth of body parts at this stage of life. Withers height was more closely related to rump height (r = 0.97 in both age groups). Shoulder width was more highly associated with rump width compared to other morpho-structural measurements (r = 0.97 and 0.75 respectively). The varying estimates of correlation in both age groups could be attributed to the fact that postnatal growth does not take place proportionally in all tissues categories and body regions; instead, it gives preference in the different growth phases to particular tissue types or body regions within those tissue categories (*Kallweit, 1993*).

Table 2. Correlation matrix for the linear body measurement of Yankasa sheep according to age group*

	WH	RH	BL	HG	TL	FAL	SW	HW	RW	EL	FL	HL	RL
WH		0.97	0.89	0.89	0.68	0.80	0.76	0.59	0.63	0.57	0.90	0.88	0.87
RH	0.97		0.89	0.89	0.70	0.82	0.75	0.59	0.63	0.57	0.91	0.89	0.88
BL	0.66	0.67		0.91	0.59	0.82	0.78	0.59	0.68	0.49	0.84	0.80	0.86
HG	052	0.56	0.52		0.63	0.87	0.77	0.64	0.70	0.52	0.82	0.79	0.90
TL	0.69	0.69	0.59	0.33		0.64	0.57	0.42	0.40	0.60	0.65	0.66	0.62
FAL	0.54	0.58	0.51	0.55	0.51		0.69	0.65	0.63	0.58	0.77	0.75	0.84
SW	0.54	0.57	0.64	0.74	0.48	0.55		0.55	0.79	0.39	0.71	0.69	0.71
HW	0.61	0.60	0.48	0.36	0.50	0.45	0.50		0.46	0.46	0.64	0.55	0.63
RW	0.48	0.49	0.59	0.70	0.33	0.52	0.75	0.41		0.20	0.61	0.58	0.63
EL	0.51	0.52	0.48	0.20	0.61	0.56	0.39	0.42	0.37		0.51	0.52	0.55
FL	0.61	0.63	0.41	0.31	0.52	0.33	0.40	0.46	0.27	0.29		0.92	0.88
HL	0.72	0.73	0.53	0.46	0.57	0.47	0.50	0.47	0.40	0.41	0.86		0.84
RL	0.70	0.71	0.58	0.51	0.55	0.55	0.60	0.50	0.49	0.44	0.50	0.57	

*Significant at P< 0.05 for all correlation coefficients.
Above diagonal: <15.5 months old sheep.
Below diagonal: 15.5 – 28 months old sheep.
WH: Withers height; RH: Rump height; BL: Body length; HG: Heart girth; TL: Tail length; FAL: Face length; SW: Shoulder width; HW: Head width; RW: Rump width; EL: Ear length; FL: Foreleg length; HL: Hind leg length; RL: Rump length.

Principal component matrix

Using principal component analysis, morphometric traits were aggregated into groups. Factor pattern coefficients of the varimax rotated factors are shown in Table 3. In < 15.5 months old sheep, four factors which contributed to 89.27% of the variability of the original thirteen traits were extracted. The first factor (principal component) accounted for 73.03% of the total variance. It was characterized by high positive loadings (factor–variate correlations) on withers height, rump height, body length, heart girth, face length, foreleg length, hind leg length and rump length; and appeared to be an index of general size factor. Similarly, *Salako (2006)* reported that 67.7% of the generalized variance in the body measurements of 0 – 14 months old Uda sheep was elucidated by the first factor. In another investigation, *Sadek et al. (2006)* found that a major size component was best represented by the first factor in stallions.

The subsequent factors in the present study were mutually orthogonal to the first and presented patterns of variation independent of general size, thereby breaking collinearity common in the analysis of closely related conformation traits. The second factor determined by shoulder width and rump width (flesh dimensions) explained only 7.61% of the generalized variance. The third factor was related to tail length and ear length and accounted for 4.73% of the total variation; whereas the fourth factor was influenced primarily by head width explaining only 3.90% of the variation. Using factor analysis, *Riva et al. (2004)*, suggested that an improvement of rump dimensions may be considered as a selection criterion. In sheep that were 15.5 – 28.3 months old, three factors were retained. The first factor which explained 57.03% of the generalized variance was associated with withers height, rump height, foreleg length, hind leg length and rump length. About 11% of the total variance was explained by the second factor which had its loadings for body length, heart girth, shoulder width and rump width. In the third factor accounting for 7.48% of the variance, the largest coefficient was for ear length, followed by tail length and face length. This is consistent with the report of *Tabachnick and Fidell (2001)* that a large number of observed variables can be reduced to a smaller number of factors.

The aggregation of conformation traits into principal components might be related to the different association of each measurement with skeletal growth, environmental influence or the maturity period, which depended on the age of the animals. There is also the possibility that the traits strongly associated with each factor are under the same gene action (pleiotropism). The exploitation of the multivariate techniques especially the principal components have been found useful for a quantitative measure of animal conformation which is desirable as it will enable reliable genetic parameters for these traits to be estimated and permits its inclusion in breeding programmes (Ibe, 1989; Mavule et al., 2012; Silva et al., 2013). Therefore, the seven extracted principal components in the present study could be used for selection of animals in order to obtain the desired body

conformation. Similarly, Kashiwamura *et al*. (2001) reported that selection of animals for potential performance ability should be guided by attention to the general balance of body comformation; which could be facilitated using principal components rather than the focus on any particular dimensional proportion of the body. Performance test traits had also been analyzed in 3 and 4–year old mares using principal components (Posta *et al*., 2007).

Table 3. Eigenvalues and share of total variance, factor and factor loadings after rotation of the body dimensions of Yankasa sheep of two age groups

	<15.5months old sheep				15.5 – 28.3 months old sheep		
Traits	Factor 1	Factor 2	Factor 3	Factor 4	Factor 1	Factor 2	Factor 3
Wither height	0.81	0.36	0.34	0.21	0.69	0.35	0.49
Rump height	0.82	0.35	0.35	0.19	0.69	0.38	0.48
Body length	0.74	0.46	0.23	0.27	0.36	0.55	0.46
Heart girth	0.70	0.47	0.28	0.33	0.26	0.87	0.04
Tail length	0.41	0.31	0.77	0.02	0.49	0.15	0.69
Face length	0.59	0.40	0.38	0.41	0.17	0.51	0.59
Shoulder width	0.43	0.77	0.26	0.20	0.27	0.82	0.26
Head width	0.32	0.24	0.20	0.85	0.44	0.30	0.45
Rump width	0.35	0.87	0.02	0.17	0.11	0.86	0.22
Ear length	0.27	-0.02	0.83	0.34	0.13	0.12	0.89
Fore leg length	0.84	0.29	0.27	0.25	0.92	0.11	0.10
Hind leg length	0.84	0.27	0.30	0.14	0.86	0.27	0.20
Rump length	0.78	0.33	0.28	0.32	0.50	0.46	0.41
Eigenvalues	9.49	0.99	0.62	0.51	7.41	1.39	0.97
Percentage of total variance	73.03	7.61	4.73	3.90	57.03	10.70	7.48

Conclusion

The principal component analysis technique explored the interdependence in the original thirteen body shape characters by analyzing them simultaneously rather than individually. The allocation of the body measurements to factors (principal components) was age dependent. In <15.5 months old sheep, four factors which accounted for 89.27% of the generalized variance were extracted. However, three factors which explained 75.21% of the total variation were identified in 15.5 – 28.3 months old sheep. Since variation in flesh dimensions (shoulder width and rump width) was not associated with body height, selection could be geared

towards the improvement of body shape in order to obtain a tall animal with characteristic meat animal traits.

Analiza glavnih komponenti osobina konformacija ovaca rase Yankasa

A. Yakubu

Rezime

Ova studija je obavljen u cilju procene biometrijskih osobina 227 ovce rase Yankasau severnoj Nigeriji korišćenjem multivarijacionog pristupa. Telesme mere koje su razmatrane u ispitivanju su sledeće: visinagrebena, visina krsta, telesna dužina, obim srca, dužinarepa, dužinalica, širina ramena, širina glave, širina sapi, dužina uha,dužinaprednjih nogu, dužinazadnjih nogu i dužina krsta. Životinje su bile podeljene u dve starosne grupe: <15,5 i 15,5 - 28,3 meseci, respektivno. Opšti linearni model je korišćen za proučavanje uticaja starosne grupe, a faktorska analiza glavnih komponenti je obavljena da se definiše oblik tela prema korelacionoj matrici trinaest telesnih mera. Starosna grupa je značajno($P<0.05$) uticala na morfološkeosobine osim dužine ušiju. Pirsonovi koeficijenti korelacije su pozitivni i značajni u obe starosne grupe. U u grupi ovacauzrasta <15,5 meseci, četiri glavne komponente (faktori) su izdvojene (odnos varijanse = 89.27).Prvi faktor čini 73,03% od ukupne varijanse i bio protumačen kao mera opšte veličine.Drugi faktor koji je objasnio 7,61% od generalizovane varijanse pokazuje tendenciju da opiše dimenzije tela (širinaramena i krsta), dok jetreći faktor imao uticaj na dužine repi ušiju.Četvrti faktor je uticao na širinuglave. U grupi ovaca starosti 15.5-28.3 meseci, tri faktora (odnos varijanse = 75.21) su identifikovani. Ovih sedam se mogu uzimati u obzir u programima oplemenjivanja da poboljšaju usaglašenost tela ovaca, jer razlika u osobinama mesa nije povezana sa telesnom visinom.

References

BROWN J.E., BROWN C.J., BUTTS W.T. (1973): Evaluating relationships among immature measures of size, shape and performance of beef bulls I. Principal components as measures of size and shape in young Hereford and Angus bulls. Journal of Animal Science, 36, 1010.

HERRERA M., RODERO E., GUITERREZ M.J., PENA F., RODERO J.M. (1996): Application of multifactorial discriminant analysis in the morpho-structural differentiation of Aadalusian caprine breeds. Small Ruminant Research, 22, 39–47.

IBE S.N. (1989): Measures of size and conformation in commercial broilers. Journal of Animal Breeding and Genetics, 106, 461–469.
JOHNSON R.A., WICHERN D.W. (2002): Applied Multivariate Statistical Analysis. 5th Ed. Prentice Hall, New Jersey. Pp 767.
KALLWEIT E. (1993): Methodical development of growth analysis up to magnetic resonance imaging. Animal Research and Development, 37, 77-93.
KESKIN S., DASKIRAN I., KOR A. (2007): Factor analysis in a multiple linear Regression model for the prediction of carcass weight in Akkeci kids. Journal of Applied Animal Research, 31, 201-204.
KASHIWAMURA F., AVGAANDORJ A., FURUMURA K. (2001): Relationships among body size, conformation, and racing performance in Banei draft racehorses. Journal of Equine Science, 12, 1-7.
MAVULE B.S., MUCHENJEB V., BEZUIDENHOUT C.C., KUNENE N.W. (2012): Morphological structure of Zulu sheep based on principal component analysis of body measurements. Small Ruminant Research, http://dx.doi.org/10.1016/j.smallrumres.2012.09.008.
NGERE L.O., ADU I.F., OKUBANJO I.O. (1984): The indigenous goats of Nigeria. Animal Genetic Resources Information, 3, 1-9.
NUGENT R.A., NOTTER, D.R. (1991): Body measurements of crossbred calves sired by Simmental bulls divergently selected for progeny first-calf calving ease in relation to birth weight. Journal of Animal Science, 69, 2422-2433.
POSTA J., KOMLOSI I., MIHOK S. (2007): Principal component analysis of performance test traits in Hungarian sport horse mares. Arch. Tierz Dummerstorf, 50, 125 – 135.
RIVA J., RIZZI R., MARELLI S., CAVALCHINI L.G. (2004): Body measurements in Bergamasca sheep. Small Ruminant Research, 55, 221-227.
SADEK M.H., AL-ABOUD A.Z., ASHMAWY A.A. (2006): Factor analysis of body measurements in Arabian horses. Journal of Animal Breeding, 123, 369-377.
SALAKO A.E. (2006): Principal component factor analysis of the morphostructure of immature Uda sheep. International Journal of Morphology, 24, 571–574.
SALHAB S.A., ZARKAWI M., WARDEH M.F., AL-MASRI M.R., KASSEM R. (2001): Development of testicular dimensions and size, and their relationship toage, body weight and parental size in growing Awassi ram lambs. Small Ruminant Research, 40, 187-191.
SHAHIN K.A., SOLIMAN A.M., MOUKHTAR A.E. (1995): Sources of shared variability for the Egyptian cattle body shape (conformation). Indian Journal of Animal Science, 65, 759–764.
SHAHIN K.A. (1996): Selection Indexes using live measurement or their varimax rotated factors for improving meat weight distribution: Application on carcasses of Pekin ducks. Arch Geflugelkd, 60, 103–108.
SILVA M.C., LOPESA F.B., VAZ C.M.S., PAULINIC F., MONTESINOSA I.S., FIORAVANTIA M.C.S., MCMANUS C., SERENO J.R.B. (2013): Morphometric

traits in Crioula Lanada ewes in Southern Brazil. Small Ruminant Research, 110, 15-19.
SPSS. (2001): Statistical Package for Social Sciences. SPSS Inc., 444 Michigan Avenue Chicago, IL 6064.
TABACHNICK B.G., FIDELL L.S. (2001): Using Multivariate Statistics. 4th Ed., Allyn and Bacon, New York, U.S.A. Pp. 966.
WILSON R.T., DURKIN J.W. (1984): Age at permanent incisor eruption in indigenous goats and sheep in semi-arid Africa. Livestock Production Science, 11, 451–455.
YAKUBU, A., MOHAMMED G. L. (2012): Application of path analysis methodology in assessing the relationship between body weight and biometric traits of Red Sokoto goats in Northern Nigeria. Biotechnology in Animal Husbandry, 28, 107-117.
YAKUBU A., SALAKO A.E., ABDULLAH A-R. (2011): Varimax rotated principal component analysis of the zoometrical traits of Uda sheep. Archivos de Zootecnia, 60, 813-816.
YAKUBU A., AKINYEMI, M.O. (2010): An evaluation of sexual size dimorphism in Uda sheep using multifactorial discriminant analysis. Acta Agriculturae Scandinavica A- Animal Science, 60, 74-78.
YAKUBU A. (2003): Phenotypic variation in the body measurements of Uda sheep. M.Sc. Thesis, Department of Animal Science, University of Ibadan, Ibadan. Pp.64.
YAKUBU A., ABDULLAH A.R., ARI M.M., OGAH D.M. (2005): Studies on live weight and linear body measurements of West African Dwarf sheep in North Central Nigeria. Production Agriculture and Technology, 1, 137-145.

Received 2 February 2013; accepted for publication 19 March 2013

Biotechnology in Animal Husbandry 29 (1), p 75-82 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.06:636.5'64
DOI: 10.2298/BAH1301075R

HERITABILITY, PHENOTYPIC AND GENETIC CORRELATIONS OF THE GROWTH INTENSITY AND MEAT YIELD OF PIGS

Č. Radović[1], M. Petrović[2], B. Živković[1], D. Radojković[2], N. Parunović[3], N. Brkić[4], N. Delić[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
[4]Ministry of Agriculture, Forestry and Water Management - Directorate for Water, 11000, Belgrade, Republic of Serbia
Corresponding author: cedomirradovic.izs@gmail.com
Original scientific paper

Abstract: The five year study included two genotypes of gilts of performance tested gilts, Swedish landrace and crosses F_1 generation SLxLY. Of total number (n=3600), 1709 animals were genotype SL and 1891 animals were genotype SLxLY. Measuring of back fat thickness in the loin part (FT1), between 3rd and 4th lumbar vertebrae, 7cm laterally to the back line; back fat thickness (FT2) and depth of the *musculus longissimus dorsi* (MLD) between the 3rd and 4th rib from the rear, 7cm laterally to the back line. Assessment of meat yield was done using the ultrasonograph apparatus Piglog 105. In regard to meat yield indicators, medium heritability values were established for FT1 h^2=0.461, and high values for FT2 h^2=0.639, and for meat yield h^2=0.633. Low heritability was established for depth of MLD (h^2=0.105), life daily gain (h^2=0.110) and age at the end of test (h^2=0.103). Established phenotypic correlations between fat thickness FT1 and FT2 were strong (r_p=0.638); between fat thickness and meat yield very strong (r_p=-0.880 to -0.895), and between fat thickness and MLD very weak and negative (r_g=-0.103 to -0.216). Genetic correlations were stronger than phenotypic, so between fat thickness FT1 and FT2 the correlation was complete/full (r_g=0.930), also between fat thickness and meat yield (r_g=-0.979 to -0.982), whereas the correlation between fat thickness and MLD was strong and negative (r_g=-0.627 to -0.653). Heritability values for fat thickness and meat yield show that these traits have high level of heritability and are transfered to the progeny, whereas the level and strength of their dependance show that by decreasing the fat thickness positive influences is exhibited on meat yield, and that by increasing of depth of MLD also the meat yield is increased.

Key words: gilt; genotypes; backfat thickness; lean meat content; Piglog 105

Introduction

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. The rate of selection progress depends on the intensity of selection, heritability (h^2) or accuracy in the evaluation of the animal's breeding value and average time interval between generations, i.e. average age of parents at birth of their progeny. Heritability coefficients for growth traits and carcass side quality are medium to high (*Lo et al., 1992; Knapp et al., 1997; Hermesch et al., 2000; Chan et al., 2002; Gorjanc et al., 2003; Radović et al., 2003; Petrović et al., 2006*). In the study of five breeds at the age of 180 days and average body weight of 110 kg, *Szyndler-Nedza et al. (2010)* have established the heritability for daily gain in boars of 0.070 for Puławska breed to 0.578 for Pietrain breed, whereas for lean percentage they have established lower heritability values of 0.013 for Puławska and 0.453 for Duroc breeds. In the same study, for gilts, established values h^2 for daily gain ranged from 0.079 to 0.585, and for lean percentage, from 0.032 to 0.303. *Groeneveld and Peškovičova (1999)*, in their evaluation of the breeding value of gilts and boars in Slovakia, have established slightly lower heritability values for daily gain in the test (0.13 to 0.19). Authors state that the reason for low heritability values is in the structure of the data base.

In light of above mentioned, the need for continuous investigation and monitoring of the growth intensity and meat yield indicators is apparent, in order to determine as precisely and accurately possible the heritability, genetic and phenotypic correlations, since most of quantitative traits are influenced by numerous genes on different loci.

Material and Methods

Study of performance tested gilts was done during the period 2007 to 2001. In regard to test years, the distribution of gilts was following: n_{2007}=682, n_{2008}=875, n_{2009}=962, n_{2010}=697 and n_{2011}=384 gilts. Investigation included two genotypes – Swedish Landrace and crosses of F_1 generation SLxLY (the first one designated is the dam). Of total number of animals (n=3600) included in the study, 1709 animals were genotype SL and 1891 animals of genotype SLxLY. Measuring of the back fat thickness and depth of the *musculus longissimus dorsi* (MLD) was done on animals of body weight of 90 to 110 kg, where 60 and 80 animals were tested in each group. Ultrasonographic apparatus (Piglog 105) was used, and the anatomic locations were: fat thickness in the loin part (FT1), between 3rd and 4th lumbar

vertebrae, 7cm laterally to the back line; back fat thickness (FT2) between the 3rd and 4th rib, from the rear, 7cm laterally to the back line and MLD depth, between 3rd and 4th rib from the rear, 7cm laterally to the back line. Processing of data was done by implementation of adequate programme, i.e. use of the method of least squares (*LSMLMW and MIXMDL-Harvey, 1990*).

Traits were analised by following mixed model of the least squares:

$Y_{ijklm} = \mu + O_i + R_j + GR_k + GM_l + e_{ijklm}$

in which: .

Y_{jjklm} = demonstration of the trait of m- individual, daughter of i- sire, j- race, born k- year and measured l- year; μ= overall mean, O_i= random influence of i- sire, R_j= fixed influence of j- race, GR_k= fixed infuence of k- year of birth, GM_l= fixed influence of l- year of measurement, eijklm= random error.

Coefficient of heritability Heritability (h^2) was calculated by the method of interclass correlation of halfsisters by sires through the following formula:

$$h^2 = 4\frac{\sigma^2_{bgs}}{\sigma^2_{bgs} + \sigma^2_{igs}}$$

where:

h^2= heritability, σ^2_{bgs} = varians between gropus sires, σ^2_{igs} = varians inside groups sires.

Genetic correlations show the connection between additive effects of genes which influenced on demonstration of two traits, and we calculated it by the formula:

$$r_G = \frac{Cov}{\sqrt{\sigma^2_{bgs} \times \sigma^2_{igs}}}$$

where:

r_G= genetic correlation, Cov= covarians, σ^2_{bgs} = varians between gropus sires, σ^2_{igs} = varians inside groups sires.

Result and Discussion

Heritability values and heritability errors for growth intensity and meat yield in performance test gilts during five year research (2007. to 2011.), are presented in table 1. Presented data show that the low heritability was established for the age at the end of test (h^2=0.103) and life daily gain (h^2=0.110). In regard to meat yield indicators, determined heritability values ranged from low to high, e.g. for MLD depth low heritability was established (h^2=0.105), medium heritability value for FT1 (h^2=0.461) and high for FT2 (h^2=0.639) and lean percentage (h^2=0.633).

Table 1. Heritability (h^2) and heritability errors (Sh^2) for growth intensity and meat yield indicators

Traits	h^2	Sh^2
Age at the end of test (AET), days	0.103	0.033
Life daily gain (LDG), g	0.110	0.035
FT1, mm	0.461	0.104
FT2, mm	0.639	0.134
MLD, mm	0.105	0.033
Meat yield, %	0.633	0.133

Phenotypic correlations for growth intensity traits and indicators of meat yield are presented in table 2. Obtained results for phenotypic correlations show that the correlation between FT1 and FT2 was strong (r_p=0.638). Correlation between the fat thickness values (FT1 and FT2) and lean percentage was negative and very strong (r_p=-0.880 and r_p=-0.895), whereas weak correlation (r_p=0.332) was determined between MLD depth and % of meat. After testing of the significance of correlations according to table values it was established that they were highly significant (P<0.01).

Table 2. Coefficients of phenotypic (rp) and genetic (rg) correlations for growth intensity and meat yield indicators

Traits	rp	rg
FT1 : FT2	0.638 **	0.930 **
FT1 : MLD	-0.103 **	-0.653 **
FT1 : Meat yield	-0.880 **	-0.979 **
FT1 : AET	-0.027 ns	0.226 **
FT1 : LDG	0.021 ns	-0.252 **
FT2 : MLD	-0.216 **	-0.627 **
FT2 : Meat yield	-0.895 **	-0.982 **
FT2 : AET	-0.019 ns	0.267 **
FT2 : LDG	0.013 ns	-0.292 **
MLD : Meat yield	0.332 **	0.692 **
MLD : AET	-0.015 ns	-0.167 **
MLD : LDG	0.016 ns	0.171 **
Meat yield : AET	0.023 ns	-0.262 **
Meat yield : LDG	-0.016 ns	0.286 **
AET : LDG	-0.994 **	-0.977 **

Genetic correlations for traits of growth intensity and indicators of meat yield are presented in table 2. Based on presented data it is apparent that the level of dependance between FT1 and FT2 full/complete (r_g=0.930). Correlation

between back fat thickness (FT1 and FT2) and depth of MLD was negative and strong (r_g=-0.653 and r_g=-0.627). Negative and full/complete correlation between back fat thickness (FT1 and FT2) and lean percentage (r_g=-0.979 i r_g=-0.982), and strong correlation between MLD depth and % of meat (r_g=0.692) were established. For genetic correlations, after testing of significance of said traits, it was established that they were statistically highly significant (P<0.01), so it can be concluded that there was full/complete and strong correlation between indicators of meat yield.

Results of this research were in concordance with heritability values (Table 1) for daily gain established by *Groeneveld and Peškovičova (1999)* and *Petrović et al. (2002)* of 0.13 to 0.19. Compared to our study, slightly higher heritability values for AET in the range from 0.26 to 0.32 were established by *Li and Kennedy (1994)* and *Groeneveld et al., (1996)*. Also, *Dufek and Buchta (1987)* have established high heritability values for LDG in Large Yorkshire and Landrace (h^2=0.728 and 0.643, respectively). Our study was in concordance with results obtained by *Ducos et al. (1993)*, *Li and Kennedy (1994)* and *Tomka et al. (2010)* who have established h^2values for back fat thickness of 0.46 to 0.64. Lower h^2 values for FT1 and FT2 have been established by *Petrović et al. (2002)* and *Apostolov (2009)* of 0.18 to 0.44. Results of our study in regard to meat yield are in concordance with results of *Ducos et al. (1993)* who have established heritability value of 0.60 to 0.65 for French Large Yorkshire and Landrace, as well as results of *Groeneveld et al. (1996)* who have established in Hungarian population of large Yorkshire and Landrace heritability values of 0.66 and 0.62. Slightly lower heritability coefficients for carcass lean percentage has been established by *Petrović et al. (2002)* and *Radović et al. (2003)*, of 0.560 and 0.502. Contrary to our research, *Groeneveld et al. (1998)* have established lower heritability values for % of meat (established using apparatus PIGLOG 105) of 0.25 to 0.36 and for % of meat (determined by dissection) in the range from 0.25 to 0.39. *Szyndler-Nędza et al. (2010)* have established for gilts of Polish large Yorkshire and Polish Landrace breeds lower heritability values h^2 for back fat thickness (0.117 to 0.169) and share of meat (0.097 to 0.185) compared to present results. Heritability values established for MLD depth in animals of Polish Landrace breed (0.105:0.158) were in concordance with our results, whereas for Polish Large Yorkshire breed significantly lower h^2 value was established (0.045).

Results of our study are in concordance with studies conducted by numerous authors who have established between back fat thickness and daily gain very strong and weak genetic and phenotypic correlations (*Bereskin and Frobish, 1982; Dufek and Buchta, 1987; Skorupski et al., 1996; Senčić et al., 1999*). Also, in concordance with our results, *Dufek and Buchta (1987)* and *Li and Kennedy (1994)* have established very strong and weak phenotypic and genetic correlations between back fat thickness and AET, in the range from -0.04 to -0.12 for phenotypic, and from -0.06 to -0.17 for genetic correlations. *Suzuki et al. (2005)* have established weak genetic correlation between daily gain (of 30-105 kg) and

back fat thickness measured in the centre of the back (r_g=0,34), whereas in present study very weak negative correlation between fat thickness (FT2) and LDG (r_g=-0.292) was established. In the research by *Sonessona et al. (1998)*, less strong negative correlation was established (r_g=-0,77) between back fat thickness and meat yield compared to present result (r_g=-0,98). *Apostolov (2009)* indicates less strong negative correlations between FT1 and share of meat r_p=-0.658 and r_g=-0.723 compared to results obtained in the present study. In said research, differences established for correlation between FT2 and share of meat, in comparison to present study, were lower (r_p=-0.789 : r_P=-0.895 i r_g=-0,928: r_g=-0.982).

Conclusions

Based on these results we can conclude that the degree of heritability for age and daily gain is slightly lower than the results of other authors, which can be explained by the fact that the tests were performed on different breeds and swine herds, as well as that different models were applied, and different fixed and random factors included. Heritability values for fat thickness and meat yield show that these traits have high level of heritability and are transfered to the progeny, whereas the level and strength of their dependance show that by decreasing the fat thickness positive influences is exhibited on meat yield, and that by increasing of depth of MLD also the meat yield is increased.

Acknowledgements:

Research was financed by the Ministry of Education, Science and Technological Development of Republic of Serbia, project TR 31081.

Heritabilitet, fenotipske i genetske korelacije intenziteta porasta i mesnatosti svinja

Č. Radović, M. Petrović, B. Živković, D. Radojković, N. Parunović, N. Brkić, N. Delić

Rezime

Petogodišnjim istraživanjem su bila obuhvaćena dva genotipa nazimica švedski landras i melezi F_1 generacije ŠLxVJ. Od ukupnog broja (n=3600) istraživanjem je obuhvaćeno 1709 grla genotipa ŠL i 1891 grla genotipa ŠLxVJ. Merenje debljine leđne slanine i dubine *musculus longissimus dorsi* (MLD) i procena mesnatosti je obavljeno ultrazvučnim aparatom Piglog 105. Za pokazatelje mesnatosti utvrđeni

su srednji heritabiliteti za SL1 h^2=0,461, a visoki za SL2 h^2=0,639, i za mesnatost h^2=0,633. Nizak heritabilitet utvrđen je za dubinu MLD-a (h^2=0,105), životni dnevni prirast (h^2=0,110) i za uzrast (h^2=0,103). Utvrđene fenotipske korelacije između debljine slanine SL1 i SL2 su jake (r_p=0,638); između debljine slanine i mesnatosti korelacije su vrlo jake (r_p=-0,880 do -0,895), dok su između debljine slanine i MLD-a jako slabe i negativne (r_g=-0,103 do -0,216). Genetske korelacije su jače od fenotipskih, tako da je između debljine slanine korelacija potpuna (r_g=0,930), kao i između debljine slanine i mesnatosti (r_g=-0,979 do -0,982), dok je korelacija jaka i negativna između debljine slanine i MLD-a (r_g=-0,627 do -0,653).

References

APOSTOLOV A. (2009): Evaluation of some more important phenotypic and genetic parameters of the performance traits of small populations from the Danube White breed. Bulgarian Journal of Animal Science, 15, 471-474.

BERESKIN B., FROBISH L. T. (1982): Carcass and related traits in Duroc and Yorkshire pigs selected for sow productivity and pig performance. Journal of Animal Science, 55, 554-564.

CHAN D. E., WALKER N. P., MILLS W. E. (2002): Prediction of Pork Quality Characteristics Using Visible and Near-Infrared Spectroscopy. Transactions of the ASAE, 45, 5, 1519-1527.

DUCOS A., BIDANEL J. P., DUCROCQ V., BOICHARD D., GROENEVELD E. (1993): Multivariate restricted maximum likelihood estimation of genetic parameters for growth, carcass and meat quality traits in French Large White and French Landrace pigs. Genetics Selelection Evolution, 25, 5, 475-493.

DUFEK J., BUCHTA S. (1987): Biometric analysis of the production and reproduction characteristics of pigs kept in the elite herds in the Czech Socialist Republic and the deretmination of selection indices. Scientia Agriculture Bohemoslovaca,19, 3, 179-190.

GORJANC G., MALOVRH Š., KOVAČ M., GLAVAČ-VNUK M., ZRIM J. (2003): Proučavanje možnosti vključivte klavnih lastnosti v napoved plemenske vrednosti pri prašičih. Zbornik Biotehniške Fakultete Univerze v Ljubljani, Kmetijstvo Zootehnika, 82, 2, 89-96.

GROENEVELD E., CSATO L., FARKAS J., RADNOCZI L. (1996): Joint genetic evaluation of field and station test in the Hungarian Large White and Landrace populations. Archiv für Tierzucht, 39, 5, 513-531.

GROENEVELD E., WOLF J., WOLFOVA M., JELINKOVA V., VECEROVA D. (1998): Estimation of genetic parameters for Czech pig breeds using a multitrait animal model. Zuchtungskunde, 70, 2, 96-107.

GROENEVELD E., PEŠKOVIČOVA D. (1999): Simultaneous estimation of the covariance structure of field and station test traits in Slovakian pig populations. Czech Journal of Animal Science, 44, 145-150.

HARVEY R.W. (1990): User's guide for LSMLMW and MIXMDL. Ver. PC–2, 1–91.

HERMESCH S., LUXFORD B.G., GRASER H.U. (2000): Genetic parameters for lean meat yield, meat quality, reproduction and feed effciency traits for Australian pigs. 1. Description of traits and heritability estimates. Livestock Production Science, 65, 239-248.
KNAPP P., WILLAM A., SÖLKNER J. (1997): Genetic parameters for lean meat content and meat quality traits in different pig breeds. Livestock Production Science, 52, 69-73.
LI X. W., KENNEDY B. W. (1994): Genetic parameters for growth rate and backfat in Canadian Yorkshire, Landrace, Duroc and Hampshire pigs. Journal of Animal Science, 72, 1450-1454.
LO L. L., MCLAREN G. D., MCKEITH K. F., FERNANDO L. R., NOVAKOFSKI J. (1992): Genetic Analyses of Growth, Real-Time Ultrasound, Carcass, and Pork Quality Traits in Duroc and Landrace Pigs: II. Heritabilities and Correlations. Journal of Animal Science, 70, 2387-2396.
PETROVIĆ M., RADOJKOVIĆ D., ROMIĆ D., PUŠIĆ M., MIJATOVIĆ M., BRKIĆ N. (2002): Genetska i fenotipska varijabilnost osobina performans testiranih nerastova i nazimica. Biotechnology in Animal Husbandry, 18, 5-6, 67-72.
PETROVIĆ M., PUŠIĆ M., RADOJKOVIĆ D., MIJATOVIĆ M., RADOVIĆ Č., ŽIVKOVIĆ B. (2006): Fenotipska i genetska varijabilnost osobina kvaliteta polutki i mesa. Biotechnology in Animal Husbandry, 22, 5-6, 1-10.
RADOVIĆ Č., PETROVIĆ M., JOSIPOVIĆ S., ŽIVKOVIĆ B., KOSOVAC O., FABJAN M. (2003): Uticaj različitih genotipova, očeva i sezone klanja na klanične osobine svinja. Biotechnology in Animal Husbandry, 19, 1-2, 11-16.
SENČIĆ D., ANTUNOVIĆ Z., PERKOVIĆ A. (1999): Expression of Large White young boars fattening in a performance test. Czech Journal of Animal Science, 44, 55-59.
SKORUPSKI M. T., GARRICK D. J., BLAIR H. T. (1996): Estimates of genetic parameters for production and reproduction traits in three breeds of pigs. New Zealand Journal of Agricultural Research, 39, 3, 387- 395.
SONESSON K. A., DE GREEF H. K., MEUWISSEN E. T. H. (1998): Genetic parameters and trends of meat quality, carcass composition and performance traits in two selected lines of large white pigs. Livestock Production Science, 57, 23–32.
SUZUKI K., KADOWAKI H., SHIBATA T., UCHIDA H., NISHIDA A. (2005): Selection for daily gain, loin-eye area, backfat thickness and intramuscular fat based on desired gains over seven generations of Duroc pigs. Livestock Production Science, 97, 193-202.
SZYNDLER-NĘDZA M., TYRA M., RÓZYCKI M. (2010): Coefficients of heritability for fattening and slaughter traits included in a modified performance testing method. Annals of Animal Science, 10, 117–125.
TOMKA J., PEŠKOVICOVÁ D., KRUPA E., DEMO P. (2010): Genetic analysis of production traits in pigs measured at test stations. Slovak Journal of Animal Science, 43, 2, 67 - 71.

Received 29 January 2013; accepted for publication 18 March 2013

Biotechnology in Animal Husbandry 29 (1), p 83-91 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.06:636.082'65
DOI: 10.2298/BAH1301083D

ROOSTER BODY WEIGHT INFLUENCE ON THE REPRODUCTIVE PERFORMANCE OF THE BROILER PARENTS

V. Djermanovic[1], S. Mitrovic[1], V. Djekic[2]

[1]University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Zemun, Republic of Serbia.
[2]Center for Small Grains, Save Kovacevic 31, 34000 Kragujevac, Republic of Serbia.
Corresponding author: djermanovic@agrif.bg.ac.rs
Original scientific paper

Abstract: In this research influence of the rooster body weight on reproductive performance of broiler parents was examined for Ross 308 and Cobb 500 hybrids. At the beginning of the productive cycle (24 weeks of age) for roosters Ross 308 hybrids average body weight of 3,030.00 g has been determined, while for Cobb 500 rooster average body weight was 3,045.00 g. In the 42nd week of age (middle of productive cycle), body weight of Ross 308 roosters was 4,306.00 g and 4,323.00 g for Cobb 500 roosters, while at the end of productive cycle in the 61st week of age Ross 308 hybrids had average 4.908,00 g and Cobb 500 had 4,918.50 g. Determined differences in body weight of roosters (15.00 g, 17.00 g and 10.50 g) in specific periods of productive cycle, as well as difference in body weight for the entire productive cycle (19.97 g) were not statistically significant ($P>0.05$). Between rooster body weight and fertilized eggs laying intensity positive statistically significant ($P<0.001$; $P<0.01$; $P<0.05$) correlation coefficients were determined. Between rooster body weight and hatchability percentage of the chicks positive statistically significant ($P<0.001$; $P<0.01$; $P<0.05$) correlation coefficients were determined for both hybrids. However, based on correlation coefficient it has been determined that rooster body weight had positive influence on laying intensity of fertilized eggs till 58th week of age (Ross 308) and 60th week of age (Cobb 500), while on hatchability of chicks it had positive influence till 58th week of age for both hybrids.

Key words: roosters, body weight, reproductive performance, broiler parents.

Introduction

Keeping and utilizing the parental flock, as well as incubating the planting eggs are highly specific and complex phases in production process. One has to bear

in mind that average production of one broiler parent flock, planting eggs production and day old chick production in practice is a combined result of genetic potential of the breed and breeding technology as well as the result of the egg incubation technology.

Moreover, poultry reproduction is very specific biologic process. In order to hatch a chick out of fertilized egg, it is necessary to provide needed conditions for the embryo to develop and grow. Therefore, nutrition and proper breeding conditions have influence on the poultry reproductive process. Only with proper diet, chicks breeding technology and proper utilization of the parental flock the maximal hatchability, needed chick vitality, and quality of the hatched chicks will be achieved. In order to prolong production of the fertilized eggs (day old chicks production), it is necessary to keep the roosters in constant breeding condition, where special attention should be given to the body weight. Uniformity of the flock in the terms of body weight is especially significant factor in the last weeks of the production cycle.

Next to the optimal gender aspect and age of broiler parents, on egg fertility and hatchability, body weight has significant influence *(Celeghini et al., 2001; McDaniel et al., 2004; Djermanovic, 2010; Djermanovic et al., 2009; Mitrovic et al., 2010)*. Proper hormonal functioning of the endocrine system of the laying hens and roosters is significantly dependant on the age and body development *(Wilson et al., 1979; Renden and Pirson, 1982; Bramvell et al., 1996; McGary et al., 2002; Bowling, 2003)*. At optimal body weight and specific age, laying hens ovaries are stimulated, ripening of the egg cells is being speeded up, while for roosters better ejaculate with larger number of active, movable and vital spermatozoids is being created, it has higher volume and proper pH value *(El Sahn, 2007; Gebriel et al., 2009; Abd El Ghany et al., 2011; Udeh et al., 2011; Makhafola et al., 2012a; Makhafola et al., 2012b; Orunmuyi et al., 2013)*, therefore the percent of fertilized eggs is being increased. This shows that on egg fertility and chick hatchability male and female units have equal influence. Roosters are responsible for “real” fertilization while laying hens influence the number and the chick hatchability percent from the fertilized eggs *(Djermanovic, 2010; Djermanovic et al., 2009; Mitrovic et al., 2010)*.

Body weight of the breeding birds, next to the other factors, especially age, has direct influence on laying intensity and hatchability percent from the number of fertilized eggs. Rooster breeding value is directly conditioned with the average body weight in specific age (week of the production process). Therefore, special attention is given to the influence of the body weight of roosters on the basic reproductive indicators during the production cycle. Main aim of this research was to determine what type of influence body weight of roosters has on the laying intensity of the fertilized eggs and at the hatchability percent.

Material and Methods

In the present research, two parental flocks of heavy hybrids Ross 308 and Cobb 500 were taken. During the production technology recommended by selection office was used. Broiler parents of both flocks were kept on the floor with deep bedding, diet; watering, airing and illumination were automatically controlled. Effective floor surface per facility was approximately 900 m^2, where density of population was approximately 6 birds/m^2 of floor surface.

Researched broiler parents flocks were bred till 61st week of age, both flocks started laying eggs at the beginning of the 22nd week. Eggs that were laid from the 24th week and till the end of the production cycle, were used for incubation, because at that week they were at proper minimal weight for incubation (>50.00 g). This shows that period of egg production (production of day old broiler chicks) lasted 38 weeks (from 24th till 61st week of age of broiler parents).

As starting experimental material total number of 5200 birds of both genders of Ross 308 hybrid and 5430 broiler parents of Cobb 500, bred in two separate facilities, were used. First facility was populated with 4750 ♀ and 450 ♂ Ross 308 hybrids, and second facility was populated with 4960 ♀ and 470 ♂ Cobb 500 hybrids, so that gender ration was 1:10.56 (Ross 308) and 1:10.55 (Cobb 500).

In preparation period from 21st till 24th week of age mortality and elimination for Ross 308 roosters was 4 units (0.89%), and with Cobb 500 3 units (0.64%). This means that at the beginning of the use of incubation eggs, Ross 308 broiler parents had 446 roosters, and Cobb 500 had 467 roosters.

In order to control the body weight, every week body weight of 80 roosters was individually taken using random sample method (40 roosters of Ross 308 and Cobb 500 hybrids each). By this inspection uniformity of roosters of researched flocks during production cycle and the influence of rooster body weight on reproductive indicators of broiler parents was tested.

Basic data processing was conducted using usual variation – statistical methods, and testing of the differences between hybrids was done using the T- test. For all monitored indicators average value, random sampling error and standard deviation were calculated. Determined results were used to calculate the correlation of researched indicators per week of production by using the correlation analysis. Statistical data processing was done using Analyst Program SAS/STAT *(SAS Institute, 2000).*

Results and Discussion

Average values, variability and significance in weight difference of the roosters (g) in specific periods of the production cycle and for the entire period of incubation egg production are displayed in table 1.

Table 1. Average values, variability and significance in weight difference of the roosters (g)

Production cycle period	Weeks of age (production)	Hybrid	$\bar{x}$ ±SEM	S	$\bar{d}$
Beginning	24 (1)	Ross 308	3.030,00±26.23	165.92	15.00 ns
		Cobb 500	3.045,00±27.84	176.11	
Middle	42 (19)	Ross 308	4.306,00±38.33	253.90	17.00 ns
		Cobb 500	4.323,00±40.26	254.58	
End	61 (38)	Ross 308	4.908,00±60.42	382.17	10.50 ns
		Cobb 500	4.918,50±61.90	391.52	
Entire production cycle	61 (38)	Ross 308	4.058,75±52.33	322.61	19.97 ns
		Cobb 500	4.078,72±56.19	346.43	

Data from table 1 shows that average body weight for roosters of both researched hybrids, compared to technologic normative *(www.rossbreeders.com; www.cobb-vantress.com)*, at the beginning of the production cycle was significantly lower, and at the end of the production cycle insignificantly lower (within the limits of the technologic normative). However, even beside mentioned deviation considering the rooster body weight, differences between researched hybrids were not statistically significant (P>0.05), for the specific production periods as well as for the entire usage period.

Djermanovic (2010), Djermanovic et al. (2009) and *Mitrovic et al. (2010)* determined similar results during the productive cycle with slightly higher body weights of the roosters. Moreover, significantly higher body weight of Ross hybrid broiler parents bred till 71st week of age, in specific phases of productive cycle was determined by *Celeghini et al. (2001)*: between 24th and 27th week of age (3.152 kg), 40th and 43rd week (4.990 kg), and between 60th and 63rd week of age (5.333 kg). Slightly lower average body weight for Ross hybrid roosters (4.27 kg) during the middle of the production cycle (18 weeks of production) was determined by *McDaniel et al. (2004).*

For analyzed parent flocks, next to the determined variations for rooster body weight, with aim to have better overview of body weight influence on reproductive performance, phenotype correlation coefficients between monitored indicators were calculated (table 2).

Up to 50th week of age for Ross 308 hybrid and 53rd week of age for Cobb 500 parental flocks, statistically positive (P<0.001; P<0.01; P<0.05) correlation coefficient was determined between body weight and laying intensity of fertilized eggs (table 2). From the data in table 2 it is noticeable that from 51st week of age (Ross 308) and 54th week of age (Cobb 500), weak, very weak or no correlation was determined between rooster body weight and laying intensity of fertilized eggs and determined correlation was not statistically significant (P>0.05).

Table 2. Phenotype correlation between rooster body weight, laying intensity (%) of the fertilized eggs (r_1) and hatchability (%) of the chicks from fertilized eggs (r_2)

Weeks of age (production)	Ross 308			Cobb 500		
	Body weight, g	r_1	r_2	Body weight, g	r_1	r_2
41 (18)	4220,00	$0,673^{***}$	$0,880^{***}$	4223,00	$0,737^{***}$	$0,882^{***}$
42 (19)	4306,00	$0,642^{**}$	$0,859^{***}$	4323,00	$0,704^{***}$	$0,873^{***}$
43 (20)	4335,00	$0,614^{**}$	$0,826^{***}$	4381,50	$0,670^{***}$	$0,863^{***}$
44 (21)	4495,00	$0,575^{**}$	$0,777^{***}$	4498,00	$0,631^{**}$	$0,840^{***}$
45 (22)	4495,00	$0,539^{**}$	$0,735^{***}$	4493,50	$0,599^{**}$	$0,809^{***}$
46 (23)	4500,00	$0,508^{**}$	$0,689^{***}$	4523,00	$0,570^{**}$	$0,768^{***}$
47 (24)	4507,50	$0,475^{*}$	$0,645^{***}$	4552,00	$0,541^{**}$	$0,717^{***}$
48 (25)	4546,00	$0,443^{*}$	$0,593^{***}$	4574,50	$0,514^{**}$	$0,643^{***}$
49 (26)	4594,00	$0,407^{*}$	$0,544^{**}$	4602,00	$0,486^{**}$	$0,560^{**}$
50 (27)	4599,00	$0,381^{*}$	$0,476^{**}$	4625,35	$0,458^{*}$	$0,480^{**}$
51 (28)	4610,50	$0,336^{ns}$	$0,420^{*}$	4634,00	$0,429^{*}$	$0,406^{*}$
52 (29)	4645,00	$0,298^{ns}$	$0,358^{ns}$	4667,00	$0,399^{*}$	$0,345^{ns}$
53 (30)	4668,00	$0,262^{ns}$	$0,303^{ns}$	4680,00	$0,369^{*}$	$0,289^{ns}$
54 (31)	4682,00	$0,228^{ns}$	$0,253^{ns}$	4692,50	$0,338^{ns}$	$0,241^{ns}$
55 (32)	4735,00	$0,186^{ns}$	$0,193^{ns}$	4742,50	$0,300^{ns}$	$0,192^{ns}$
56 (33)	4750,00	$0,133^{ns}$	$0,140^{ns}$	4755,00	$0,260^{ns}$	$0,149^{ns}$
57 (34)	4800,50	$0,083^{ns}$	$0,084^{ns}$	4807,50	$0,213^{ns}$	$0,097^{ns}$
58 (35)	4842,50	$0,027^{ns}$	$0,017^{ns}$	4846,50	$0,159^{ns}$	$0,041^{ns}$
59 (36)	4884,00	$-0,028^{ns}$	$-0,069^{ns}$	4890,00	$0,093^{ns}$	$-0,037^{ns}$
60 (37)	4902,50	$-0,085^{ns}$	$-0,163^{ns}$	4911,00	$0,025^{ns}$	$-0,134^{ns}$
61 (38)	4908,00	$-0,140^{ns}$	$-0,251^{ns}$	4918,50	$-0,050^{ns}$	$-0,226^{ns}$

However, in the last three weeks (59^{th}, 60^{th} and 61^{st}) with Ross 308, and 61^{st} week of age for Cobb 500 negative correlation was determined, but it wasn't statistically significant ($P>0.05$). Unlike for laying intensity of fertilized eggs, between rooster body weight and chick hatchability positive statistically significant ($P<0.001$; $P<0.01$; $P<0.05$) correlation was determined up to 51^{st} week of age for both hybrids (table 2). For both parent flocks from 52^{nd} till 58^{th} week of age (29^{th} till 35^{th} week of production) positive phenotype correlation, between monitored indicators, was determined but it wasn't statistically significant ($P>0.05$). Moreover, in the last three weeks of production cycle, for both broiler parents flocks, rooster body weight had negative influence on hatchability of the chicks, but determined phenotype correlation between monitored indicators was not statistically significant ($P>0.05$). Based on results, it can be noted that rooster body weight had positive influence on laying intensity of fertilized eggs up to 58^{th} week of age for Ross 308 hybrid and up to 60^{th} week of age for Cobb 500 hybrid, and it had positive influence on hatchability of day old broiler chicks up to 58^{th} week of age for both researched parent flocks.

Djermanovic (2010) had similar results regarding the rooster body weight influence on reproductive traits in his research of the reproductive performances of

two broiler parent flocks (Ross 308 and Cobb 500), whereas *McGary et.al. (2002)* had similar results with two different strains of different age (strain A – 50 weeks and strain B – 48 weeks). Similar research was conducted by *Wilson et al. (1979)* and *Bowling (2003)* where authors are pointing out that rooster fertility, due to larger number of abnormal spermatozoids, decreases with the increase of age, therefore the body weight of males decrease too. Authors determined negative correlation between growth rate and fertility of the roosters. *Bowling (2003)* found negative correlation (r = -0.23) between body weight of young (35 – 45 weeks) and older (50 – 65 weeks) roosters and fertility, whereas *Wilson et al. (1979)* determined weak correlation (r = -0.39 do r = 0.09). However, *El Sahn (2007), Gebriel et al. (2009), Abd El Ghany et al. (2011), Udeh et al. (2011)* and *Orunmuyi et al. (2013)* determined positive correlation between body weight of different genotype roosters and volume, concentration of ejaculate, while *Makhafola et al. (2012a)* and *Makhafola et al. (2012b)* points out that body weight of Naked Neck roosters and Ovampo genotypes is in negative correlation with volume, concentration and pH value of the sperm, and for the autochthonous South African strain Potchfstroom Koekoek the correlation was positive. In the contrary of the above stated, *Renden and Pirson (1982)* and *Bramvell et al. (1996)* determined that there is no difference in fertility of the young (39 weeks) and old (65 weeks) roosters, therefore pointing out that spermatozoids retain their physiological ability to fertilize in at least two productive cycles.

Conclusion

Based on obtained results it can be concluded that average body weight for both researched hybrids, compared to technologic normative, was lower at the beginning as well as in the end of the productive cycle. However, even with variations in specific periods, differences between rooster body weight of the researched hybrids were not statistically significant ($P>0.05$).

In weekly observation, between average body weight of the roosters and laying intensity of fertilized eggs positive statistically significant ($P<0.001$; $P<0.01$; $P<0.05$) correlation was determined up to the 50^{th} week of age (27^{th} production week) for Ross 308 hybrid, and for the Cobb 500 hybrid up to the 53^{rd} week of age (27^{th} production week). Between rooster body weight and hatchability percent positive statistically significant ($P<0.001$; $P<0.01$; $P<0.05$) correlation was determined up to the 51^{st} week of age (28^{th} production week) for both researched hybrids.

Based on obtained phenotype correlation coefficients and their significance it can be said that rooster body weight has had a significant influence on breeding ability, because in the last three weeks of the production cycle, for both flocks, between researched indicators especially egg fertilization, negative correlation coefficients were determined. This leads to the fact that with increase of body

weight breeding ability of the roosters declines, which points out the requirement to significantly lessen the production cycle time.

Acknowledgement

The credit for making this research possible goes to the Ministry of Education and Science of the Republic of Serbia for sponsoring part of the study within project No TR – 31033.

Uticaj telesne težine petlova na reproduktivne performanse brojlerskih roditelja

V. Djermanovic, S. Mitrovic, V. Djekić

Rezime

Ispitivanje uticaja telesne težine petlova na reproduktivne performanse brojlerskih roditelja sprovedeno je kod hibrida Ross 308 i Cobb 500. Na početku proizvodnog ciklusa (24. nedelja starosti) kod hibrida Ross 308 utvrđena je prosečna telesna težina petlova 3.030,00 g, a Cobb 500 3.045,00 g. U 42. nedelji starosti (sredina proizvodnog ciklusa) telesna težina petlova iznosila je 4.306,00 g (Ross 308) i 4.323,00 g (Cobb 500), dok je na kraju proizvodnog ciklusa (61. nedelja starosti) telesna težina petlova kod hibrida Ross 308 iznosila 4.908,00 g, a hibrida Cobb 500 4.918,50 g. Utvrđene razlike telesne težine petlova (15.00 g, 17.00 g i 10.50 g) u određenim periodima proizvodnog ciklusa, kao i razlika u telesnoj težini petlova za ceo proizvodni ciklus (19.97 g), nisu bile statistički signifikantne ($P>0.05$). Između telesne težine petlova i intenziteta nosivosti oplođenih jaja utvrđeni su pozitivni statistički značajni ($P<0.001$; $P<0.01$; $P<0.05$) koeficijenti korelacije do 50. nedelje starosti kod hibrida Ross 308, odnosno 53. nedelje kod roditeljskog jata hibrida Cobb 500, a između telesne težine petlova i procenta izvodljivosti pilića utvrđeni su pozitivni statistički značajni ($P<0.001$; $P<0.01$; $P<0.05$) koeficijenti korelacije do 51. nedelje starosti kod oba ispitivana hibrida. Međutim, na osnovu koeficijenata korelacije utvrđeno je da je telesna težina petlova pozitivno uticala na intenzitet nosivosti oplođenih jaja do 58. nedelje starosti (Ross 308), tj. 60. nedelje starosti (Cobb 500), a na izvodljivost jednodnevnih brojlerskih pilića do 58. nedelje starosti kod oba ispitivana roditeljska jata.

References

ABD EL GHANY F.A., ALM EL DEIN A.K., SOLIMAN M.M., REZZA A.M., EL-SODANY S.M. (2011): Relationship between some body measurements and fertility in males of two local strains of chickens. Egyptian Poultry Science, 32(II), 331-349.

BOWLING E.R. (2003): Sperm mobility in broiler breeders. Master of Science, Athens, The University of Georgia.

BRAMWELL R.K., McDANIEL C.D., WILSON J.L., HOWARTH B. (1996) Age effects of male and female broiler breeders on sperm penetration of the perivitelline layer overlying the germinal disc. Poult. Sci., 75: 755-762.

CELEGHINI E.C.C., ALBUQUERQUE R., ARRUDA R.P., LIMA C.G. (2001): Seminal characteristics evaluation of the male broiler breeder selected by comb development to reproduction. Braz. J. Vet. Res. Anim. Sci., v. 38, n. 4, 177-183.

DJERMANOVIC V. (2010): Phenotype variability and correlation of productive and reproductive characteristics of heavy hybrid hen lines Cobb 500 and Ross 308. PhD thesis, University of Belgrade, Faculty of Agriculture.

DJERMANOVIC V., MITROVIC S., DJEKIC V., RAJOVIC M., RAKIC S. (2009): Efficiency of use genetic potential of broiler poultry parents in our country. Poultry, 7/8, 29-39.

EL-SAHN A.A. (2007): Use of phenotypic traits to predict cocks fertility, 2. The ornamental and non-ornamental traits. Egyptian Poultry Science, 27, 1085-1097.

GEBRIEL G.M., KALAMAH M., EL-FIKY A., ALI A.F.A. (2009): Some factors affecting Semen quality trait in Norfa cocks. Egyptian Poultry Science, 29(11), 677-693.

MAKHAFOLA M.B., UMESIOBI D.O., MPHAPHATHI M.L., MASENYA M.B., NEDAMBALE T.L. (2012a): Characterization of Sperm Cell Motility Rate of Southern African Indigenous Cockerel Semen following Analysis by Sperm Class Analyser. Journal Animal Science Advances, 2(4), 416-424.

MAKHAFOLA M.B., UMESIOBI D.O., NEDAMBALE T.L. (2012b): Relationship between phenotypic and sperm traits of South African indigenous cockerels. International Journal of Livestock Production, Vol. 3(6), 61-65.

McDANIEL C.D., HOOD J.E., PARKER H.M. (2004): An Attempt at Alleviating Heat Stress Infertility in Male Broiler Breeder Chickens with Dietary Ascorbic Acid. International Journal of Poultry Science, 3 (9): 593-602.

McGARY S., ESTEVEZ I., BAKST M.R., POLLOCK D.L. (2002): Phenotypic Traits as Reliable Indicators of Fertility in Male Broiler Breeders. Poultry Science, 81: 102–111.

MIROVIC S., DJERMANOVIC V., RADIVOJEVIC M., RALEVIC N., OSTOJIC Dj. (2010): Possibilities of more efficient usage of genetic potential of broiler breeders. African Journal of Biotechnology, Vol. 9 (18), 2584-2594.

ORUNMUYI M., AKANWA C.L., NWAGU B.I. (2013): Semen Quality Characteristics and Effect of Mating Ratio on Reproductive Performance of Hubbard Broiler Breeders. Journal of Agricultural Science, Vol. 5 (1), 1916-9760.
RENDEN J.A., PIERSON M.L. (1982): Long-term reproductive performance of broiler breeder males selected for semen performance. Poult. Sci., 61:1214-1217.
SAS INSTITUTE (2000): SAS (Statistical Analysis System). User's guide: Statistics. SAS Institute Inc. Cary, NC.
UDEH I., UGWU S.O.C., OGAGIFO N.L. (2011): Predicting Semen traits of Local and Exotic Cocks using Linear Body Measurements. Asian Journal of Animal Sciences, 5 (4): 268-276.
WILSON H.R., PIESCO N.P., MILLER E.R., NESBETH W.G. (1979): Prediction of the fertility potential of broiler breeder males. World's Poult. Sci. J., 35:95-118.
www.rossbreeders.com
www.cobb-vantress.com

Received 25 February 2013; accepted for publication 15 March 2013

Biotechnology in Animal Husbandry 29 (1), p 93-104 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.085.2:636.087.8'65
DOI: 10.2298/BAH1301093A

PERFORMANCE AND NUTRIENT DIGESTIBILITY IN BROILER CHICKS AS INFLUENCED BY MULTI-ENZYME ADDITION TO STARTER DIETS CONTAINING PALM KERNEL MEAL

V. E. Aya[1*], B. A. Ayanwale[2], A. T. Ijaiya[2] and A. Aremu[2]

[1]Department of Animal Science, College of Agriculture Lafia, Nigeria
[2]Department of Animal Production, Federal University of Technology Minna, Nigeria
*Corresponding author: ayabuga@gmail.com.
Original scientific paper

Abstract: This study was conducted to investigate the performance and nutrient digestibility in broiler chicks as influenced by multi-enzyme (Hemicell +Roxazyme G) addition to starter diets containing palm kernel meal. Nine experimental diets were formulated such that diet 1 which served as control contained 0 % PKM without enzyme supplementation. Diet 2, 3, 4 and 5 contained 10, 20, 30 and 40 % PKM levels respectively with multi-enzyme supplementation while diets 6, 7, 8 and 9 contained 10, 20, 30 and 40 % PKM inclusion levels respectively without multi-enzyme supplementation. Five hundred and forty (540) day old hybro broilers of mixed sex in ratio (1: 1) were randomly assigned to nine diets in a completely randomized design. Each treatment was replicated thrice with 20 birds per replicate. The experiment lasted 35 days. The results showed that nutrient digestibility in the control and 10 % PKM with enzyme supplementation were similar but were significantly ($P<0.05$) higher than other PKM diets with or without supplementation. There was significant ($P<0.05$) improvement in body weight and body weight gain and reduce feed intake with supplementation. Birds fed with 20 % PKM with enzyme showed similarity with control birds in all the performance parameters measured. Enzyme addition significantly ($P<0.05$) reduced cost of feed consumed at 30 % level of inclusion with PKM while cost per kilogram weight gain and cost of production were lower at 20 % PKM level.

Key words: broiler chicks, palm kernel meal, multi-enzyme supplementation

Introduction

At the most basic level feedstuff consists of protein, starch, fat and fibre. In monogastric animal the fibre component has been considered to be wasted and in

some instances compound called non-starch polysaccharide (NSP) can exert anti-nutritive activity on the animal. β-Mannan and non-starch polysaccharide are the main fibre component of Palm kernel meal (PKM) (*Sundu et al., 2006; Esuga et al., 2008*), such component are not easily digested by poultry (especially chicks). The anti-nutritional effect of these NSPs is manifested by poor growth accomplished by depressed nutrient utilization (*Annison and Choct, 1991*). These adverse effects can be overcome by dietary supplementation of exogenous enzyme (*Sultan, 2008*).

There are many enzymes available in the market. In practical poultry feeding, the choice of appropriate enzyme for a particular diet is important while it is not definitely known which enzyme will be better for PKM but initially the choice depends on the NSP content. *Duad et al. (1993)* reported that the PKM cell wall comprised 58 % mannan, 12 % cellulose and 4 % xylan. From the composition of carbohydrate of PKM, *Sundu et al. (2006)* suggested that three enzyme; mannanase, α-galactosidase and cellulase may be needed to breakdown the main polysaccharides component. *Alemawor et al. (2009)* recommended the use of a combination of various fibrolytic enzymes activities to enhance saccharification of NSPs. *Fischer (2003)* had indicated that birds at young age had their performance and digestibility improve largely due to improvement in viscosity reduction as consequence of enzyme addition.

This study was design to investigate the effect of supplementation of PKM based diets with mixture of enzyme preparation, Hemicell® and Roxazyme G® containing mannanase, α-galactosidase and cellulase as well as glucanase and xylanase for their effects on chick performance.

Materials and Methods

Experimental diets

Nine broiler starter diets representing 9 dietary treatments were formulated to be isocaloric and isonitrogenous diets with 3000 kcal/Kg ME and 22 % crude protein respectively. The starter phase lasted for 5 weeks. The control with 0 % PKM and no enzyme supplementation consisted of a basal diet with maize and soyabean as major sources of energy and protein respectively. Diets 2, 3, 4, 5 contained 10, 20, 30 and 40 % PKM levels respectively supplemented with multi-enzyme supplementation while diets 6, 7, 8, 9 contained 10, 20, 30 and 40 % PKM inclusion levels respectively without multi-enzyme supplementation as shown in Table 1.

Table 1. Composition of experimental diets containing exogenous enzyme

Ingredients(%)	**Dietary Palm kernel meal levels (%)**								
	With enzyme supplementation					**without enzyme supplementation**			
	0	**10**	**20**	**30**	**40**	**10**	**20**	**30**	**40**
Maize	61.50	54.00	45.50	37.00	27.10	54.00	45.50	37.00	27.10
Soybean meal	31.00	28.60	26.60	24.60	23.60	28.60	26.60	24.60	23.60
Palm oil	0.50	0.50	1.00	1.50	2.40	0.5.00	1.00	1.50	2.40
PKM	0.00	10.00	20.00	30.00	40.00	10.00	20.00	30.00	40.00
Bone Meal	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
Limestone	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50	1.50
Salt	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Premix*	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Methionine	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Lysine	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20	0.20
Hemicell	0.00	0.05	0.05	0.05	0.05	0.00	0.00	0.00	0.00
RoxazymeG	0.00	0.02	0.02	0.02	0.02	0.00	0.00	0.00	0.00
Fish meal	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50	2.50
Total	100	100	100	100	100	100	100	100	100
Calculated values									
M.E(kcal/kg)	3005.64	2998.12	2982.02	2972.92	2972.35	2998.12	2982.02	2972.92	2972.35
Protein(%)	22.18	21.89	21.77	21.64	21.64	21.89	21.77	21.64	21.64
Fibre(%)	3.20	4.46	5.76	7.04	8.35	4.46	5.76	7.04	8.35
EE(%)	3.18	3.64	4.10	4.57	5.01	3.64	4.10	4.57	5.01
Calcium(%)	1.19	1.20	1.21	1.22	1.23	1.20	1.21	1.22	1.23
P (%)	0.60	0.61	0.61	o.62	0.63	0.61	0.61	o.62	0.63

Premix* to provide the following per kg of diet vitamin A 12500 I.U. $Vitd_3$ 2500 I.U. vit E 50 mg vit K_3 2.5mg; vit B_1 3.0mg; vit B_2 6.0mg; vit B_6 6.0mg; niacin 40.0mg; calcium pantothenote 10mg; Biotin 0.80mg; vit B_{12} 0.25mg; folic acid 1.0mg; choline chloride 300mg; manganese 100mg; iron 50mg; zinc 45mg; cobalt 0.25mg; iodine 1.55mg; selenium 0.1mg.

The multi-enzyme preparation is a combination of Hemicell® and Roxazyme G®. Hemicell® is a fermentation product of *Bacillus lentus* with β-mannanase as active ingredient. It also contains α- galactosidase. It was included at

manufacturer's recommendation level of 0.05 % or 500 g/ton. Roxazyme G® is an enzyme complex derived from *Trichoderma vivida* with glucanase cellulase and xylanase activity. The inclusion recommendation is 200 mg/kg feed. The inclusion levels of the enzyme were as recommended by their respective manufacturers.

Experimental birds and their management

A total of 540 day old hybro broilers of mixed sexes in ratio (1:1) were used for this experiment which lasted for 35 days. The birds were randomly divided into nine experimental groups in completely randomized design (CRD) that were replicated thrice with 20 birds per replicate. The experimental chicks were brooded on deep litter system. Wood shavings were used as litter materials and constant management of the litter through aeration and litter changing when wet were carried out. The main source of heat was electricity while supplementary heat was provided with stove, charcoal and kerosene lantern. There was a gradual reduction of heat from 95 to 75^0 F for the first 4 weeks. Feed and fresh clean water were provided *ad libitum*. The feeders and drinkers were allocated one each per pen of 20 birds. Antibiotics that contain vitamins and electrolytes as constituents (*Keproceryl*) were provided in the drinking water from day 1 to 7, and from 14 to 16 days, respectively. At days 10 and 21, Gumboro vaccine was orally administered on the birds while Newcastle disease vaccine (Lasota) was also orally administered when the birds were 28 days old. Oral administration of coccidiostat (Amprolium) was done on days 23 to 25. The management practices included cleaning of the feeders and water container daily; addition of fresh feed to the stale feed in the feeders were done after the litter and droppings in the feeders have been removed. Periodic turning of the feeders to ensure feeding to appetite was also done. Dead birds were removed promptly to prevent the contamination of the other birds which might peck them.

Data collection

The response parameters taken were body weight, measured individually on a weekly basis; feed consumption was recorded on a pen basis daily by finding the difference between the amount offered and the left over collected the following day (this was later expressed on a weekly basis); body weight gain was determined weekly while feed conversion ratio was calculated by dividing feed intake by weight gain.

Three birds were randomly selected from each of the three replicate groups at day 25 to conduct nutrient digestibility trial. The chickens were housed in metabolic cages for 3 days adjustment period before data collection started. Feed was allocated to all the birds on equal basis. Total droppings were collected separately for each replicate during the last 4 days of the trial as adopted by Ayanwale and Aya (2006). Proximate composition of the feacal samples was determined by A.O.A.C. (2006) methods. The percentage digestibility of the following: dry

matter, crude protein, crude fibre, lipids, total ash and NFE were computed individually using the formula adopted by Iyayi and Davis (2005), that is:

Nutrient digestibility (%) = Nutrient intake- Nutrient in feaces / Nutrient intake x 100

The prevailing price of feedstuff was used to calculate cost of formulated feed per kilogram diet. The feed intake per bird for the 5- week experimental period was used to obtain the cost of feed consumed by a bird. The cost per kilogram weight gain was calculated using the procedure of *Ukachukwu and Anugwa (1995)* by taking the product of cost per kilogram fed and feed conversion ratio of birds. The cost of production was estimated as the product per kilogram weight gain and mean total weight gain.

Proximate and data analysis

Experimental diets and feacal samples were analyzed for proximate composition according to A.O.A.C (2006) methods. Data collected were subjected to multivariate analysis of variance using the general linear model (GLM) procedure of SPSS (2001) package. Where significant differences ($P<0.05$) were found, Duncan's Multiple Range test (DMRT) was applied to separate the means.

Results and Discussion

Chemical composition

The proximate composition and metabolizable energy values of experimental diets are presented in Table 2. DM (%) and CP (%) values of the experimental diets ranged from 90.75 to 91.04 and 21.10 to 22.08 respectively.

Table 2. **Proximate composition and Metabolizable Energy values of the experimental diets**

Parameters	Palm Kernel Meal level (PKM) (%)					Enzyme supplemented Palm Kernel Meal level (EPKM) (%)			
	0	10	20	30	40	10	20	30	40
DM	90.75	90.90	90.87	90.95	91.03	90.89	90.88	90.95	91.04
CP	22.08	21.98	21.89	21.88	22.03	22.00	21.92	21.88	22.0
EE	3.71	3.93	3.88	3.94	4.08	3.94	3.88	3.92	4.10
CF	3.90	4.83	5.94	7.64	8.87	4.80	5.95	7.64	8.88
Ash	4.75	4.77	5.06	6.59	6.75	4.78	5.05	6.60	6.75
NFE	56.31	55.89	50.26	49.30	45.98	55.90	50.90	49.35	45.98
ME(Kcal/Kg)	3091.28	3073.38	3021.23	2952.35	2974.35	3073.40	3021.44	2952.35	2974.40

PKM: Palm kernel meal
EPKM: enzyme supplemented PKM

The EE (%) values in the PKM with or without enzyme supplementation ranged from 3.93 to 4.10. The PKM diets with or without enzyme had CF content ranged from 4.83 to 6.75 % while the Ash and NFE were 4.77 to 6.75 and 55.89 to 49.35 % respectively. The ME ranged from 2974.35 to 3073.40 Kcal/ kg.

The effects of PKM diets with or without enzyme treatment on nutrient digestibility is presented in Tables 3. DM and EE digestibility values of 10 % enzyme diet were significantly (P<0.05) higher than the values for the control and other PKM diets with or without enzyme treatment. However, CP, Ash and NFE digestibility values in control diet were significantly (P<0.05) higher compared to all other PKM diets with or without enzyme supplementation. The CF digestibility in control and 10 % PKM inclusion with enzyme were similar and significantly (P<0.05) higher than 20, 30 and 40 % PKM with or without enzyme supplementation. Increasing levels of PKM inclusion, significantly (P<0.05) depressed nutrient digestibility in PKM diets with or without enzyme supplementation.

Table 3. **Nutrient digestibility of broiler chickens fed palm kernel meal with exogenous enzyme supplementation at the starter phase**

Treatments		**DM %**	**CP %**	**CF %**	**EE %**	**Ash %**	**NFE %**
Diets	**Level (%)**						
Control	0	69.86^{b}	60.12^{a}	39.55^{a}	67.11^{b}	65.34^{a}	77.49^{a}
PKM	10	64.44^{c}	57.27^{c}	38.15^{b}	52.50^{c}	43.22^{d}	69.45^{d}
EPKM	10	71.18^{a}	59.45^{b}	39.91^{a}	68.05^{a}	51.18^{b}	74.53^{b}
PKM	20	59.24^{d}	54.55^{e}	37.40^{c}	53.05^{d}	42.20^{e}	67.65^{e}
EPKM	20	68.27^{c}	57.26^{c}	38.56^{b}	58.42^{c}	44.31^{c}	72.35^{c}
PKM	30	58.59^{e}	46.37^{f}	26.56^{f}	48.40^{f}	35.29^{g}	63.44^{h}
EPKM	30	64.55^{c}	55.86^{d}	33.93^{d}	53.85^{d}	40.32^{f}	69.85^{d}
PKM	40	54.01^{f}	39.33^{g}	21.32^{g}	28.38^{g}	30.29^{h}	58.37^{g}
EPKM	40	58.43^{e}	46.59^{f}	28.31^{e}	49.34^{e}	35.59^{g}	65.37^{f}
	SEM	1.07	1.31	1.24	2.12	0.18	1.05
	LS	*	*	*	*	*	*

a-g means in the same column with different superscripts differ significantly (P<0.05)
SEM standard Error of means; PKM: Palm kernel meal; EPKM: enzyme supplemented PKM; LS: level of significant; *: significant (P<0.05)

Results of performance of enzyme supplemented PKM birds during first 5 weeks (Starter phase) are presented in Table 4. All the initial body weights were similar to the values for the control and all the valued ranged from 50.23 to 50.39g. Feed intake was similar to the control in treatment with 10 % and 20 % enzyme supplemented diets but significantly (P<0.05) lower compared with other diets with or without enzyme supplementation. Feed intake increased with increasing levels of PKM inclusion with or without enzyme supplementation but enzyme supplementation reduced feed intake in each of the groups.

Table 4. **Performance of broiler chickens fed palm kernel meal diets with exogenous enzyme supplementation at the starter phase**

Treatments		Initial body wt (g)	Feed intake (g)	Final Body weight (g)	Body wt gain (g)	Feed: gain Ratio
Diets	**Level (%)**					
Control	0	50.23	2128.88^{g}	1174.31^{b}	1124.08^{b}	1.89^{e}
PKM	10	50.39	2172.13^{f}	1150.52^{b}	1100.13^{c}	1.82^{e}
EPKM	10	50.91	2113.29^{g}	1245.53^{a}	1194.62^{a}	1.77^{e}
PKM	20	50.36	2373.89^{e}	1145.48^{b}	1095.12^{c}	2.17^{c}
EPKM	20	50.36	2146.94^{fg}	1173.47^{b}	1113.11^{bc}	1.91^{de}
PKM	30	50.39	2476.43^{c}	1059.60^{c}	1009.21^{e}	2.45^{b}
EPKM	30	50.37	2434.37^{d}	1088.45^{c}	1038.08^{d}	2.35^{d}
PKM	40	50.36	2724.43^{a}	1041.67^{c}	991.31^{e}	2.78^{a}
EPKM	40	50.36	2565.54^{b}	1077.63^{c}	1027.27^{e}	2.50^{ab}
	SEM	0.74	13.74	13.24	6.03	0.17
	LS	ns	*	*	*	*

a-g: means in the same column with different superscripts differ significantly (P<0.05)
SEM: standard Error of means; LS: level of significant; ns: not significant (P>0.05); * significant (P<0.05)
PKM: Palm kernel meal; EPKM: enzyme supplemented PKM

The body weight and body weight gain of birds fed 10 % PKM enzyme treated diets were significantly (P<0.05) superior to the control. However 20 % PKM enzyme treated birds was similar to control. Enzyme supplementation improved body weight and body weight gain in 10 % EPKM compared to 10 % PKM fed broilers at starter phase. Feed: gain ratio of the birds fed 10, 20 % enzyme supplemented diets and 10 % non-enzyme diets were similar. Enzyme supplementation had no effect on the performance of birds at 40 % level of inclusion.

Effect of diets on economic of broiler chick is presented in Table 5. The feed cost per kg in the control (N80.34) was significantly (P<0.05) higher compared with 20, 30, and 40 % PKM with or without enzyme supplementation but was similar to that of 10 % PKM with or without enzyme supplementation. Feed cost per kg decreased with increase in inclusion level of PKM with or without enzyme supplementation. Cost of feed consumed in 20 % PKM with enzyme was significantly (P<0.05) lower compared to control and other PKM diets with or without enzyme supplementation. However no significant difference (P>0.05) was observed in the cost of feed consumed in control, 20 % PKM without enzyme and 30 % PKM with or without enzyme supplementation diets. Diet with 40 % PKM without enzyme had significantly (p<0.05) higher cost of feed consumed. Cost per kg weight gain and cost of production in 10 % PKM with or without enzyme and 20 % PKM with enzyme supplementation were similar but were significantly

(P<0.05) higher compared to control and other PKM diets with or without supplementation.

Table 5. **Economics of producing broiler chicks fed palm kernel meal diets with exogenous enzyme supplementation**

Treatments			feed cost (₦/kg)	Cost of feed consumed (₦)	Cost per Kg wt gain(₦)	cost of production(₦)
Diet	**Level (%)**					
Control	0		80.34^{a}	171.03^{c}	151.84^{e}	178.31^{c}
PKM	10		76.63^{ab}	166.45^{d}	139.46^{f}	162.20^{e}
EPKM	10		77.46^{ab}	163.69^{e}	137.10^{f}	168.94^{d}
PKM	20		72.30^{bc}	171.63^{c}	156.89^{d}	179.71^{bc}
EPKM	20		73.14^{b}	157.03^{f}	139.69^{f}	163.92^{e}
PKM	30		68.84^{cd}	170.48^{c}	168.65^{c}	178.70^{c}
EPKM	30		70.81^{c}	172.38^{c}	166.40^{c}	181.12^{b}
PKM	40		68.27^{d}	185.99^{a}	189.79^{a}	197.69^{a}
EPKM	40		70.73^{c}	181.46^{b}	176.83^{b}	190.56^{b}
	SEM		0.85	4.14	0.75	5.51
	LS		*	*	*	*

a-g means in the same column with different superscripts differ significantly (P<0.05)
SEM: standard Error of means. PKM: Palm kernel meal; EPKM: enzyme supplemented PKM
LS: level of significant; * significant (P<0.05)

The results of the chemical composition of the experimental diets were in closed agreement with the calculated values and were within the recommended values of 20-25 %, 4.5-5.5% and 2800-3200 Kcal/kg CP, CF and ME respectively for broilers in the tropics as reported by *Oluyemi and Roberts (2000)*. However, the fibre content of 8.87 % in the diet was slightly above the recommended values of 4.5- 5.5% CF for broilers in the tropics (*Sundu, et al., 2006*). This high level is due to the level of PKM above 10 % in the experimental diets.

The results of nutrient digestibility agreed with *Sundu et al. (2008)* that most of the dietary fibre in PKM was in the form of mannan which is indigestible by monogastric animals. The decrease in digestibility with increase in inclusion levels of PKM indicates that most of the dietary fibre in PKM could not be digested. *Sundu et al. (2008)* reported that 30 % digestibility of fibre indicated that the bird may be gaining some benefit from the source of carbohydrates. This could explain the moderate performance recorded in PKM diets without enzyme supplementation. Effect of enzyme supplementation of PKM on nutrient digestibility showed that nutrient digestibility in diets with enzyme was higher compared to diets without enzyme which could have contributed to the higher body weight gain and improved FCR observed among birds fed enzyme supplemented diets. There was a significant (P<0.05) variation in fat digestibility in all the

treatments which was higher in the control and enzyme treated diets compared to diets without enzyme supplementation. *Salih et al. (1991)* and *Moharrery, (2006)* suggested that the low lipid digestibility in broilers chicken fed diets with a high content of NSPs might be due to bacterial overgrowth in the small intestine and subsequent excessive deconjugation of bile acids, which reduced their efficacy in solubilizing lipids. *Sekoni et al (2008)* reported an improvement in the body weight and feed conversion efficiency due to an increase in fat digestibility which consequently increased bioavailability of fat soluble vitamins and protein digestibility when enzyme *maxigrain*® was fed to broilers.

The increase in digestibility of crude fibre observed with enzyme supplementation is an indication of the breakdown of the non-starch polysaccharides by the enzyme in the PKM. *Iyayi and Davis (2005); Iyayi, Ogunsola and Iyayi (2005)* and *Sekoni et al. (2008)* observed increased in crude fibre digestibility and concluded that the enzymes must have acted on cellulose, glucoronoxylans, arabinoxylans and mannan thereby reducing the crude fibre content and subsequently increased the energy contents and NFE digestibility. The degrading of mannan to mannose by enzyme probably release large amounts of soluble carbohydrate thus accounting for the high level of NFE digestibility among the enzyme treated diets compared to untreated diets.

The reduction in DM, CP, EE, Ash and NFE digestibility in the PKM diets was attributed to the effect of replacement of highly digestible carbohydrate source, maize by PKM which was of low digestibility. High fibre in the diet resulted in the increase rate of passage of the fibrous feed through the gastro-intestinal tract with a consequent reduction in the time of ingesta (in nutrient) exposure to enzymatic degradation and time of contact with the absorptive membrane as explained by *Iyayi et al. (2005)*.

Performance of birds at the first 5 weeks with or without enzyme supplementation showed increase in body weight, body weight gain, improved feed: gain ratio, PER and EE in the control and enzyme treated diets relative to diets without enzyme supplementation. This is due to enzyme effect as reported by *Choct (2006)*, who stated that when enzymes are added to high fibre monogastric diets, they cause the degradation of β- Mannan and 70 % NSPs into soluble metabolizable products for monogastric. Feed intake was decreased in the control and enzyme diets compared to diets without enzyme. *Ezieshi and Olomu (2004)* reported higher feed intake in birds fed PKM based diets compared with maize-based diets due to its faster rate of passage through the digestive tract. Feed to gain ratio was better among enzyme supplemented diets and the control compared with all other diets without enzyme supplementation. This is similar to the reports of *Atteh (2000)* and *Esuga et al. (2008)* who separately observed improvements in weight gain and feed: gain ratio in birds fed enzyme supplemented diets. *Esuga et*

al. (2008) also observed lower weight of birds fed increasing levels of PKM without enzyme supplementation.

Enzyme supplementation was able to reduce cost of feed consumed with PKM level of inclusion at 30 % while cost per kilogram weight gain and cost of production were lower at 20 % PKM with enzyme supplementation. This shows that it is more profitable and economical to supplement PKM diets for chicks with multi-enzyme. The higher feed consumption of birds fed 20, 30 40 % PKM without supplementation increased cost per kilogram and as such not recommended. These findings pointed to the role of enzymes in degrading NSPs in PKM based diets. Increased nutrient availability and metabolizable energy due to NSP degradation by enzyme could be the reason for the improvement observed.

Proizvodne performanse i svarljivost hranljivih materija kod brojlerskih pilića pod uticajem dodatka multi-enzima starter obrocima koji sadrže sačmu od palminog jezgra

V. E. Aya, B. A. Ayanwale, A. T. Ijaiya, A. Aremu

Rezime

Cilj ove studije je bio da se ispitaju performanse i svarljivost hranljivih materija kod brojlerskih pilića, pod uticajem multi-enzim aditiva (Hemicell +Roxazyme G), u starter obrocima koji sadrže sačmu palminog jezgra. Devet eksperimentalnih obroka su formulisani tako da je brok 1, koji je služio kao kontrola, sadržavao 0% PKM, bez dodatka enzima. obroci 2, 3, 4 i 5 su sadržavali 10, 20, 30 i 40% PKM, respektivno, sa multi-enzimskim dodatkom, dok su obroci 6, 7, 8 i 9 sadržavali 10, 20, 30 i 40% PKM, bez dodatka multi-enzima. Hibro brojleri starosti petstotina četrdeset (540) dana, mešovitog pola u odnosu (1:1) su nasumično raspoređeni u devet grupa sa različitim obrocima. Svaki tretman je triput ponovljen sa 20 grla u svakom ponabljanju. Eksperiment je trajao 35 dana. Rezultati su pokazali da je svarljivost hranljivih materija u kontroli i obroku sa 10% PKM sa enzimom slična, ali je značajno ($P<0.05$) veća nego kod drugih PKM obroka, sa ili bez dodatka enzima. Utvrđeno je značajno ($P<0.05$) poboljšanje telesne mase i prinosa telesne mase, kao i smanjenje unosa hrane koja je sadržavala dodatke. Brojleri hranjeni sa 20% PKM sa enzimom pokazali su sličnost sa kontrolnim brojlerima u svim izmerenim proizvodnim parametrima. Enzim značajno ($P<0.05$) smanjuje troškove konzumirane hrane u obroku sa 30% PKM, a cena po kilogramu prirasta težine i troškova proizvodnje bila je niže kod obroka sa 20% PKM.

References

ALEMAWOR, F., DZOGBEFIA, V. P., ODDOYE, E. O. K AND OLDHAM, J. H. (2009). Enzyme cocktail for enhancing poultry utilisation of cocoa pod husk. *Scientific Research and Essay*, 4 (6): 555-559.

ANNISON, G AND CHOCT, M (1991). Antinutritional activities of cereal non starch polysaccharides in broiler diets and strategies minimizing their effects. *World poultry Science Journal*, 47: 232-241.

A.O.A.C. (2006). Association of Official Analytical Chemists. *Official Methods of Analysis.* 18th edition (W. Horwitz Editor). Washington. D.C

ATTEH, J. O. (2000). Replacement value of *nutrase xyla* supplemented wheat bran for maize in broiler diet. Paper presented at a two day seminar on starting the millennium with an array of tailor made biotechnical improver for flour milling and baking industry. Sheraton hotel Lagos. May 2-3 2000.

AYANWALE, B. A AND AYA V. E. (2006). Nutritional Evaluation of Cornflakes Waste in diets for Broilers. *Pakistan Journal of Nutrition,* 5(5): 485-489.

CHOCT, M (2006). Enzyme for the feed industry, past, present, and future. *World Poultry Science Journal,* 6: 5-15

DAUD, M. J., JARVIS, M. C AND RASIDAH, M. A (1993). Fibre of PKC and its potential as poultry feed. Animal production strategies in the challenging environment. *In*: Proceedings of the 16th Malaysian Society of Animal Production Annual Conference, Selangor Darul Ehsan, Malaysia, p. 32-36.

ESUGA, P. M., SEKONI, A. A., OMAGE, J. I AND BAWA, G. S (2008). Evaluation of enzyme (Maxigrain®) supplementation of graded levels of palm kernel meal (PKM) on the performance of broiler chickens. *Pakistan Journal of Nutrition*, 7(4) 607-613.

EZIESHI, E. V AND OLOMU, J. M (2004). Comparative performance of broiler chickens fed varying levels of palm kernel meal and maize offal. *Pakistan Journal of Nutrition*, 3(4) 254-257.

FISCHER, N. E (2003). Interrelationship of diet fibre and Endoxylanase with bacteria in the chicken gut. Ph.D. thesis, Dept of Animal and poultry science, University of Saskatchewan Saskatoon.

IYAYI, E. A., DAVIES, B. I. (2005). Effect of enzyme supplementation of palm kernel meal and brewer's dried grains on the performance of broilers. *International Journal of Poultry Science,* 4 (2): 76-80.

IYAYI, E. A., OGUNSOLA, O AND IYAYI, R. (2005). Effect of three sources of fibre and period of feeding on the performance, carcasses measure, organ relative weight and meat quality in broilers. *International Journal of Poultry Science,* 4(9): 695-700.

MOHARRERY, A. (2006). Comparison of performance and digestibility characteristics of broilers fed diets containing treated hulled barley or hulless barley. *Czech Journal Animal Science,* 51 (3): 122-131.
NIMET. (2009). Nigeria Meteorological Agency, Lafia, Nasarawa State.
OLUYEMI, J. A AND ROBERTS, F. A (2000). *Poultry production in the warm wet climate*, 2nd edition. Macmillan publishers, New Zealand.
SALIH, M. E, CLASSEN, H. L AND CAMPBELL, G. L. (1991). Response to chicken fed on hulless barley to dietary β-glucanase at different ages. *Animal Feed Science Technology,* 33:139-149.
SEKONI, A. A., OMAGE, M. J.J., BAWA, G. S AND ESUGA, P.M. (2008). Evaluation of enzyme (maxigrain®) Treatment of graded levels of palm kernel meal (PKM) on nutrient retention *Pakistan Journal of Nutrition,* 7(4): 614-619.
SOLTAN, M.A. (2009). Growth performance, Immune response and carcass traits of broiler chicks fed on graded levels of palm kernel cake without or with enzyme supplementation. *Livestock Research for Rural Development,* 21(3): 1-11.
SPSS (2001). Statistical package for the Social sciences. SPSS Inc., New York
SUNDU B., KUMAR A., DINGLE, J. (2006). Palm kernel meal in broiler diets: effect on chicken performance and health. *World Poultry Science Journal,* 62:316-325.
SUNDU, B., KUMAR, A., DINGLE, J. (2008). Amino acid digestibilities of palm kernel meal in poultry. *Journal of the Indonesian Tropical Animal Agriculture,* 33(2): 139-144.
UKACHUKWU, S. N. AND ANUGWA, F. O. I. (1995). Bioeconomics of feeding raw treated soyabean to broilers. *Nigerian Journal of Animal Production,* 27: 137-147.

Received 5 February 2013; accepted for publication 10 March 2013

Biotechnology in Animal Husbandry 29 (1), p 105-114 , 2013 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC631.563.:633.32
DOI: 10.2298/BAH1301105D

THE EFFECT OF CARBOHYDRATE ADDITIVE AND INOCULATION ON QUALITY OF RED CLOVER SILAGE

B. Dinić[1], N. Đorđević[2], D. Terzić[1], M. Blagojević[1], J. Marković[1], G. Jevtić[1], M. Vukić-Vranješ[3]

[1]Institute of Forage Crops Kruševac, 37251 Globoder, Serbia
[2]University of Belgrade, Faculty of Agriculture, Zemun, Serbia
[3]Institute of science application in agriculture, Belgrade, Bulevar Despota Stefana 68b, Beograd,
Corresponding author: bora.dinic@ikbks.com
Original scientific paper

Abstract: In this experiment, wilted masses of red clover of cultivar K-17 from the first cut was ensiled in three treatments: a) no additives, b) with the addition of corn (6% of biomass) and c) with the addition of inoculant BioStabil Plus. The experiment design was according to the method of a completely random plan (single factorial trial) in triplicates. Based on the results it can be concluded that the wilted biomass of red clover can be successfully ensiled without additives. However, the inoculation of red clover biomass achieves the most favourable pH value (4.20), the lowest level of degradation of the protein expressed in the amount of NH_3-N (107.7 gkg^{-1} N), the largest production of lactic acid (91.3 gkg^{-1} DM) and acetic acid (42.6 gkg^{-1} DM), in the absence of butyric acid. Adding maize meal in the amount of 6% contributed to somewhat more favourable fermentation and increase of the energy value of silage. When using the DLG and Weissbach methods for assessing the quality of silage, all silages were classified into the first class. Contrary to this, according to the Zelter method, control and inoculated silages were evaluated as class III, because of the large amounts of acetic acid. In practices inoculants based on homo-and hetero-fermentative bacteria of lactic acid fermentation are recommended for use, because the increased production of acetic acid contributes positively to the aerobic stability of silage.

Key words: silage, red clover, corn meal, inoculant, quality

Introduction

The use of conserved forages (hay and silage) throughout the year, combined with the required amount of concentrate, is generally accepted and widespread trend in countries with developed cattle breeding. The fact that the quality hay

depends on weather conditions caused the hay to be the feed of the most variable chemical composition and nutritional value (*Antov et al., 1994*). Methods of mechanical conditioning of biomass accelerate storage (drying) of hay (*Savoie et al., 1997*), and the use of chemical preservatives (propionic acid-based) enables storing of the mass with higher moisture content (*Bolsen, 1993*). Nevertheless, the most effective way to preserve maximum nutritional value of plant mass of the first cut is ensiling. In this regard, in Serbia there are numerous experiences with alfalfa, while the ability of red clover ensiling was far less studied. Although it is superior forage crop to alfalfa due to its remarkable biological properties, red clover is a significant potential for food production in areas with unfavourable pH (*Dinić et al., 2012*).

Conservation of biomass of red clover by method of ensiling, based on lactic-acid fermentation, is difficult because of the low content of fermentable carbohydrates, high buffer value and high moisture content (*Beyer et al., 1982*). These problems are solved, similar to other legumes, by wilting, using carbohydrate supplements/additives, and by using the bacterial inoculants (*Đorđević and Dinić, 2003*). However, the knowledge and experience gained in experiments on alfalfa, can not be used directly in ensiling of red clover, which is specific in regard to certain characteristics of chemical composition (*Đorđević et al., 2012*).

The practice of ensiling requires maximum simple, cheap and effective procedures. Accordingly this experiment is planned with the aim to investigate the effect of the use of carbohydrate supplement (corn meal) and bacterial inoculant on chemical composition and quality parameters of red clover silage.

Material and Methods

In the experiment, the biomass of wilted red clover of the first cut, cultivar K-17 at the start of flowering phase was ensiled. The average dry matter content at ensiling time was 331.7 g kg-1. The experiment was set up as a random plan (single factorial trial) with three replications: a) control (RC_C - Red Clover control), b) the addition of 6% of corn (RC_{+MM} - Red Clover + maize meal) c) addition of inoculant Biostabil Plus (RC_{+I} - Red Clover + inoculant). Plus BioStabil is the inoculant of the Austrian company Biomin containing homo-fermentative lactic acid bacteria (*Enterococcus faecium, and Bacillus plantarum*) and hetero-fermentative lactic acid bacteria (*Bacillus brevis*) at a concentration of 5×10^{10}cfu per gram. Ensiling was carried out in experiment containers holding 130 dm^3.

Chemical analyses of samples of initial material, maize meal and silage were conducted in the laboratory of the Institute of forage crops in Kruševac, according to standard methods (*AOAC, 2002*). Parameters of biomass suitability for ensiling (mono saccharides, soluble carbohydrates and buffer capacity) were determined by the method of *Weissbach (1967).* Minimum content of DM which guarantees

obtaining of stable silage without the presence of butyric acid was calculated according to the formula Y (g/kg) = 450 – (80 × S/BC) by *Beyer et al. (1982).*

In the biomass of red clover, maize meal and silage the following were analytically determined: dry matter (DM), crude protein (CP), crude fibre (CF), crude fat (CL), NDF, ADF, ash, Ca and P, and nitrogen-free extracts (NFE) were calculated. The silage DM content was determined, the degree of acidity (pH), ammonia nitrogen (NH_3-N), the content of acetic, butyric and lactic acid. In the assessment of silage quality three methods were used: DLG, Zelter and Weissbach (*Đorđevic and Dinić, 2003*). Nutritional value expressed in units of NE_L and NE_M was calculated according to *Obračević (1990),* and digestibility coefficients of nutrients according to *Glamočić (2002).* On the basis of chemical composition (CP, ADF, NDF) and dry matter digestibility, the relative feed value (RFV) was obtained according to the standards of quality for legumes and grass - American Forage and Grassland Council (*Schroeder, 1994*). The results of chemical analysis were analyzed by variance analysis, and statistical significance of differences was tested by LSD test (*StatSoft, 2006*).

Results and Discussion

Suitability of biomass for ensiling

Suitability of plants for silage can be decided on the basis of the ratio of sugar quantity and buffer capacity (*Dinić et al., 1998*). If the sugars present were used for the synthesis of lactic acid with 100% efficiency, the ratio of sugar quantity to the buffer capacity (S/BC) could be 1.0. However, the natural micro-flora can transform only about 50 % of the sugar into lactic acid, which means that the S/BC ratio must be greater than 1.0. This relationship depends on the level of dry matter in the ensiled material. In particular S/BC ratio, production of lactic acid is even greater if the dry matter content is higher (*Beyer et al.,1982*).

Suitability of biomass for silage is being tested in order to forecast the quality of silage prepared without additives. Determined content of soluble carbohydrates (sugars), substances essential for lactic acid fermentation, in the present experiment was 132 gkg^{-1}DM and buffer capacity (BC) was 59.7, while the ratio S/BC 2.23. *Weissbach (1967)* has stated that the above ratio must be greater than 3.0 in order to obtain stable silage without the presence of butyric acid. Contrary to this, the results from this experiment showed that stable and high-quality silage can be obtained under significantly narrower S/BC ratio. S/BC ratio of 2.23 requires a minimum dry matter content of 282 gkg^{-1}, while in the experiment DM of 332 gkg^{-1} was determined, i.e., was significantly higher.

Table 1. Suitability of red clover biomass for ensiling, gkg^{-1} DM

Type of material	DM, gkg^{-1}	Sugars, gkg^{-1} DM	Buffer capacity, meq/100 g DM	S/BC	Required DM, gkg^{-1}
Red clover	332	133	59,7	2,23	282
Maize meal	875	167	30,5	5,48	-

Sugar content in the biomass of red clover in this experiment was slightly higher than the results of previous research (106-124 gkg^{-1} DM), obtained by *Dinić et al. (1994),* while the values for BC were almost identical (58-60 meq/100 g DM lactic acid).

Content of nutrients in the initial material and silage

The chemical composition of the biomass of red clover and maize meal is shown in Table 2. At the time of ensiling, biomass of red clover contained 168.3 gkg^{-1}DM of crude protein and less than 250 gkg^{-1} DM crude fibre which provided good nutritional value and good digestibility. Maize meal contained higher concentration of CF (41.8 gkg^{-1} DM). Compared to the initial material, the control silage and silage with maize meal contained lower amount of CP, while the treatment with inoculants had almost the same amount of CP. Crude fat content was higher in silages compared to the initial material, which could be explained by forming of the lower fatty acids in the fermentation process (Table 2). NDF and ADF in the initial material were similar to that of the control silage, slightly lower than in silages with additives. There was no statistically significant difference in the content of NDF and ADF in silage.

Based on the content of ADF of 338.6 gkg^{-1} DM, and according to quality standards for legumes and grasses, green mass of the material was evaluated I quality class, and according to the NDF content of 482.5 gkg^{-1} DM for the same criterion it was ranked in the second quality class.

The calculated nutritional value of green mass expressed in units of NEL and NEM (Table 2) was significantly higher than the nutritional value of silage. This difference was a result of higher values of the digestibility coefficient of the green mass compared to silage.

Relative feed value (RFV) of the initial material was 120 points, 117 for the control silage and silage with maize meal 124 points, and accordingly classified as Class II quality, whereas silage with inoculants was evaluated with 130 points and evaluated as the first class quality.

Table 2. Chemical composition and nutritional value of initial material and red clover silage, gkg^{-1} DM

Sample	CP	CF	CL	NFE	NDF	ADF	NEL MJkg^{-1}	NEM MJkg^{-1}	RFV
Initial material									
Red clover	168.3	242.9	31.6	443.5	482.5	338.6	5.54	5.56	120
Maize meal	100.1	41.8	46.2	769.8	-	-			
Silage									
RC_C	156.9 ^{b}	267.6 ^{a}	40.7 ^{a}	430.0 ^{b}	491.8 ^{a}	348.2 ^{a}	5.17	5.04	117
RC_{+MM}	161.5 ^{b}	236.6 ^{b}	45.0 ^{a}	452.5 ^{a}	475.9 ^{a}	325.9 ^{a}	5.23	5.13	124
RC_{+I}	168.7 ^{a}	249.1 ^{b}	41.8 ^{a}	435.7 ^{b}	462.5 ^{a}	313.7 ^{a}	5.19	5.07	130
LSD 0,05	7.07	18.15	8.57	16.64	30.01	36.49	-	-	-
LSD 0,01	10,07	27,43	12,99	25,21	45,48	55,30	-	-	-

Differences in the contents of CL, ADF, NDF, mineral substances, Ca and P between the different treatments were not statistically significant (Tables 2 and 3). Statistical significance was found in regard to the CP content between the silage with inoculants on the one hand and silage without additives and with the addition of maize meal on the other (Table 2). Inoculant provided better preservation and less degradation of CP compared to other treatments. Control silage had significantly highest content of CF.

The amount of CP determined in this trial was lower than the previously determined value by *Dinić et al. (1994),* which amounted to 180 and 192.5 gkg^{-1} DM. The amount of CF in the experiment (about 250 gkg^{-1} DM) was higher compared to the results of *Svirskis (2005),* which were in the range 16.69-21.25% and *Đukić et al. (2007),* which amounted to 195.6 gkg^{-1} DM. Slightly lower content of CP in DM of the first cut red clover (149.1-163.1 gkg^{-1}) were determined by *Dinić (1990),* also *Vasiljević et al. (2012)* (14.9-16.5%), and significantly higher in DM of the second cut obtained by the same authors (16.1 to 18.7%). The nutritive value of the initial material and silage (contents of CP, CF, NFE, NDF, ADF, RFV, etc.) largely depend on the stage of cutting of plants, as confirmed by the research results of *Ignjatović et al. (2003), Marković et al. (2010)* and *Vasiljević et al. (2012).*

Maize meal contained a small amount of total mineral matters and calcium in relation to red clover. The Ca: P ratio in red clover DM was very unfavourable and amounted to 6:1. *Dinić et al. (1994)* have found a more favourable ratio of Ca and P (about 4.0-4.5: 1) in the initial material and in silage.

Table 3. Content of mineral substances in the initial material and red clover silages, gkg^{-1} DM

Samples	Ash	Ca	P
Initial material			
Red clover	113.7	18.1	3.0
Maize meal	19.3	2.1	3.0
Silage			
RC_C	104.8 [a]	20.6 [a]	3.3 [a]
RC_{+MM}	104.3 [a]	19.1 [a]	3.4 [a]
RC_{+I}	104.9 [a]	20.5 [a]	3.0 [a]
LSD 0,05	10.68	4.45	0.77
LSD 0,01	16.19	6.74	1.18

Biochemical changes in the silage

The amount of dry matter in all silages was higher than 300 gkg^{-1}. Differences in DM were significant between treatments, and the highest amount of DM was established for the treatment RC_{+MM}, which was contributed by the addition of maize meal (Table 4).

There was a statistically significant difference in the degree of acidity between the control silage and silages with additives, with the most favourable pH value (4.20) determined in silage with inoculants (Table 4). The amount of NH_3-N expressed in terms of total nitrogen is an indicator of protein degradation in the process of ensiling, and high significance for this parameter between the control silage and silages with additives was determined. Silage with inoculants had the lowest value for ammonium nitrogen, which can be explained by a low degree of proteolysis in the middle with the lowest pH value. The level of dry matter and pH are the most important factors that determine the intensity of proteolysis, but they cannot completely stop it (*Carpintero et al., 1979*).

Table 4. Parameters of biochemical changes in red clover silages

Treatments	DM, gkg^{-1}	pH	NH_3-N, gkg^{-1} N	Acid content in silages					
				Acetic		Butyric		Lactic	
				gkg^{-1} DM	% TA	gkg^{-1} DM	% TA	gkg^{-1} DM	% TA
RC_c	303.3c	4.67a	167.6a	34.2ab	36.31	1.7a	1.80	58.3b	61.89
RC_{+MM}	346.6a	4.37b	138.2ab	23.9b	25.34	0.4a	0.42	70.0b	74.23
RC_{+I}	323.3b	4.20b	107.7b	42.6a	31.81	0.0a	0.00	91.3a	68.19
LSD 0,05	13.98	0.172	38.3	13.98	-	1.92	-	18.83	-
LSD 0,01	21.20	0.260	58.0	21.20	-	2.92	-	28.53	-

Legend: TA – Total acids

Relative amount of lactic acid in all silages was greater than 60% (relative to the total acidity), which allows for the maximum number of points (20) according to the DGL method for evaluating of the quality. The amount of butyric acid was highest in the control silage (1.8%) and it was scored 9 points (out of a maximum 10) by DLG method. The highest absolute values for the amount of lactic acid and acetic acid (91.3 and 42.6 gkg^{-1} DM) were found in silage with inoculants (Table 4), which is a result of homo-and hetero-fermentative lactic acid bacteria from used inoculants. Increased content of acetic acid negatively affects the quality class of silage. In this experiment, it is certainly a result of the use of inoculants with hetero-fermentative bacteria of lactic acid fermentation and it is significant in a positive way due to increased aerobic stability of silage (*Hu et al., 2009*).

The results of these studies, in regard to the degree of acidity and acetic acid content were in the range of results obtained by *Dinić et al. (1994)* (pH 4.29 and the acetic Acid 8.66-9.98 gkg^{-1}). Less favourable values in this study were for the % of $\%NH_3$-N/ΣN (5.78-9.07%), and favourable results were found for the content of lactic (14.9 gkg^{-1}) and butyric acid (0.497-0.867 gkg^{-1}) with the dry matter content of 226.3-258.5 gkg^{-1}. Application of inoculant contributed to greater production of lactic and acetic acids and decreased production of butyric acid, which is consistent with research of *Đorđević et al. (2004)*.

In a more realistic assessment of the quality of silage three methods were used: DLG, Zelter and Weissbach (Table 5). According to the method of Weissbach and DLG, all silages were rated first class. When using the method according to Zelter, silages were evaluated as III class (treatments RC_C and RC_{+I}), and class II (treatment RC_{+MM}), as a result of higher content of acetic acid.

Table 5. Evaluation of the quality of silage using different methods

Treatments	DLG		Zelter		Weissbach	
	Number of points	Class	Number of points	Class	Number of points	Class
RCc	44	I	13	III	90	I
RC_{+MM}	48	I	15	II	95	I
RC_{+I}	49	I	13	III	95	I

Conclusion

Based on the established results in the experiment consisting of ensiling of wilted mass of red clover without additives and with the addition of maize meal (6%) and inoculant, the following was determined:

- Red clover wilted biomass can be successfully ensiled without additives and high quality silage can be achieved (Class I by DLG and Weissbach methods);
- Addition of maize meal in the amount of 6% contributes to somewhat more favourable fermentation and increase of the energy value of silage;

- The use of inoculants containing hetero-fermentative bacteria of lactic acid fermentation has negative impact on the quality of silage (Class III by Zelter method) but it is important for the aerobic stability of silage;

- Inoculation of silage provided the most favourable pH value (4.20), the lowest level of degradation of the protein expressed in the amount of NH_3-N (107.7 gkg^{-1} N), the highest production of lactic acid (91.3 gkg^{-1} DM) and acetic acid (42.6 gkg^{-1} DM), in the absence of butyric acid.

The general conclusion is that wilted biomass of red clover should be ensiled with the addition of appropriate inoculants.

Acknowledgment

The authors would like to thank the Ministry of Education, Science and Technological Development of the Republic of Serbia, which has funded this research as part of the project No. TR-31057.

Uticaj ugljenohidratnog dodatka i inokulacije na kvalitet silaže crvene deteline

B. Dinić, N. Đorđević, D. Terzić, M. Blagojević, G. Jevtić

Rezime

U eksperimentu je silirana provenula masa crvene dateline sorte K-17 iz prvog otkosa u tri tretmana: a) bez aditiva, b) sa dodatkom kukuruzne prekrupe (6% od biomase) i c) sa dodatkom inokulanta BioStabil Plus. Eksperiment je postavljen po metodi potpuno slučajnog plana (monofaktorijalnog ogleda) u tri ponavljanja.

Na osnovu utvrđenih rezultata može se zaključiti da se provenula biomasa crvene dateline može uspešno silirati bez aditiva. Međutim, pri inokulaciji biomase crvene deteline postiže se najpovoljnija pH vrednost (4.20), najmanji stepen degradacije proteina izražen kroz količinu NH_3-N (107.7 gkg^{-1} N), najveća produkcija mlečne kiseline (91.3 gkg^{-1} DM) i sirćetne kiseline (42,6 gkg^{-1} DM), uz istovremeno odsustvo buterne kiseline. Dodavanje kukuruzne prekrupe u količini od 6% doprinosi nešto povoljnijoj fermentaciji i povećanju energetske vrednosti silaže. Pri korišćenju DLG i Weissbach metode za ocenu kvaliteta sve silaže su svrstane u I klasu. Nasuprot tome, pri korišćenju Zelter metode, kontrolna i inokulisana silaža su ocenjene III klasom, zbog velike količine sirćetne kiseline.

Za praksu se preporučuje upotreba inokulanata na bazi homo- i heterofermentativnih bakterija mlečnokisleinskog vrenja, jer povećana produkcija sirćetne kiseline pozitivno doprinosi aerobnoj stabilnosti silaža.

References

AOAC (2002): Official Methods of Analysis of AOAC international. 17th ed. Association of Official Analytical Chemists, Washington, DC.

ANTOV G., ČOBIĆ T., KUNC V., ANTOV A., KASAPOVIĆ C. (1994): Ispitivanje gubitaka hranljivih materija lucerke u proizvodnji sena baliranjem. Savremena poljoprivreda, 6, 81-86.

BEYER M., CHUDY A., HOFFMAN B., HOFFMAN L., JENTSCH W., LUDDECKE F., SCHIEMANN R., SCHMIDT L., WEISSBACH F. (1982): Применение комплексной системы оценки кормов в растениеводстве. Таблица кормов для крупного рогатого скота. (Перевод с немецког). Колос, Москва. 209-267.

BOLSEN K. (1993): Effect of Alfa-Save treatment on dry matter digestibility and voluntary intake of alfalfa hay. Poster presentation at Alltech's 9th Annual symposium on biotechnology in the feed industry, April, Lexington, Ky.

CARPRINTERO C. M., HENDERSON A. R., McDONALD P. (1979): The effect of some pre-treatments on proteolysis during the ensiling of herbage. Grass and Forage Science, 34, 311-315.

DINIĆ B. (1990): Uticaj provenjavanja silo krme crvene deteline i konzervanasa na kvalitet silaže. Arhiv za poljoprivredne nauke, 51, 183, 235-244.

DINIĆ B., LUGIĆ Z., STOŠIĆ M., RADOVIĆ J. (1994): Uticaj provenjavanja i nivoa kukuruzne prekrupe na kvalitet silaže crvene i bele deteline. Biotehnologija u stočarstvu, 10, (3-4), 71-80.

DINIĆ B., KOLJAJIĆ V., ĐORĐEVIĆ N., LAZAREVIĆ D., TERZIĆ D. (1998): Pogodnost krmnih biljaka za siliranje. Savremena Poljoprivreda, 1-2, 154-162.

DINIĆ B., ĐORĐEVIĆ N., BLAGOJEVIĆ M., TERZIĆ D., ĐOKIĆ D. (2012):

DINIĆ B., ĐORĐEVIĆ N., LUGIĆ Z. (2012): Quality of alfalfa haylage depending on the aplication of inokulant and ground corn. Proceedings of The First International Syposium on Animal Science, November 2012, Belgrade 488-495.

ĐORĐEVIĆ N., DINIĆ B. (2003): Siliranje leguminoza (monografija). Vizartis – Beograd.

ĐORĐEVIĆ N., GRUBIĆ G., DINIĆ B., NEGOVANOVIĆ D. (2004): Uticaj inokulacije na hemijski sastav i kvalitet silaža od soje i kukuruza. Biotehnologija u stočarstvu, 20, 1-2, 141-146.

ĐORĐEVIĆ N., GRUBIĆ G., STOJANOVIĆ B., DINIĆ B., BOŽIČKOVIĆ A. (2012): Contemporary aspects of lucerne use in animal nutrition. 6th Central European Congress on Food, CEFood 2012, 23-26.06. Novi Sad, Serbia. Proceedings, 1514-1519.

ĐUKIĆ D., LUGIĆ Z., VASILJEVIĆ S., RADOVIĆ J., KATIĆ S., STOJANOVIĆ I. (2007): Domaće sorte višegodišnjih leguminoza, nastanak i kvantitativna svojstva. Zbornik radova. XI sipozijum o krmno bilju republike Srbije sa

međunarodnim učešćem. „Održivi sistem proizvodnje i iskorišćavanja krmnog bilja“. 44, 1, 7-19
GLAMOČIĆ D. (2002): Ishrana preživara (praktikum). Univerzitet u Novom Sadu, Poljoprivredni fakultet.
HU W., SCHMIDT R.J., MCDONELL E.E., KLINGERMAN C.M., KUNG L. (2009): The effect of Lactobacillus buchneri 40788 or Lactobacillus plantarum MTD-1 on the fermentation and aerobic stability of corn silages ensiled at two dry matter contents. Journal of Dairy Science, 92 ,3907–3914.
IGNJATOVIĆ S., DINIĆ B., LUGIĆ Z. (2003): Hranljiva vrednost crvene (*T. Pratense* L.) i bele deteline (*T. Repens* L.) u različitim fazama razvića u odnosu na potrebe preživara. Savremena Poljoprivreda, 52, 3-4, 125-127.
MARKOVIĆ J., ŠTRBANOVIĆ R., TERZIĆ D., POJIĆ M., VASIĆ T., BABIĆ S. (2010): Relative feed value of alfalfa (Medicago sativa L) and red clover (Trifolium pretense L) at different stage of growth. Biotechnology in Animal Husbandry, 26, 469-474
OBRAČEVIĆ Č. (1990): Tablice hranljivih vrednosti stočnih hraniva i normativi u ishrani preživara. Naučna knjiga, Beograd.
SCHROEDER JW (1994). Interpreting forage analysis. Extension dairy specialist (NDSU), AS-1080, North Dakota State University.
SAVOIE P., TREMBLAY D., LAJOIE R., ROBERGE M., LEMAY S.P. (1997): Forage maceration on a self-propelled mower: Effect of winrow deposition and inversion. Proceedings of the XVIII international grassland congress, Winnipeg, Manitoba, Saskatoon, Saskatchewan, Canada, session 14, 5-6.
STATSOFT, INC (2006): STATISTICA (data analysis software system), version 7.1.www.statsoft.com.
SVIRSKIS A. (2005): Red clover varieties for conventional and ecological farming sistems in Lithuania. Proceedings of the 13th Symposium of the EGF, Tartu, Estonia, 29/31 Aug 2005. Grassland Science in Europe, 10, 656-659
WEISSBACH F. (1967): Die Bestimung der Pufferkapazitat der Futterpflanzen und ihre Bedeutung der Vergarbarkeit, Aus: Tagungberichte Nr. 92 der Deutschen Akademie der Landwirtschafts wissen schaften zu Berlin, 211-219.
VASILJEVIĆ S., KATIĆ S., MIHAILOVIĆ V. (2012): Oplemenjivanje crvene deteline (*Trifolium pratense* L.). Zbornik referata sa 45. Savetovanja agronoma Srbije, 127-136.

Received 28 December 2012; accepted for publication 1 February 2013

Biotechnology in Animal Husbandry 29 (1), p 115-122 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 638.13:633.32
DOI: 10.2298/BAH1301115J

COLONY STRENGTH IN THE SPRING INSPECTION AND ITS IMPACT ON THE AMOUNT OF FORAGED POLLEN AT THE TIME OF RED CLOVER POLLINATION

G. Jevtić [1*], B. Anđelković [1], Z. Lugić [1], N. Nedić [2], K. Matović [3]

[1] Institute for forage crops Kruševac, 37251 Globoder, Republic of Serbia
[2] Faculty of Agriculture, Nemanjina 6, 11080, Belgrade-Zemun, Republic of Serbia
[3] Institute of veterinary "Kraljevo", Žička br. 34, 36000 Kraljevo, Republic of Serbia
Corresponding author: goran.jevtic@ikbks.com
Original scientific paper

Abstract: In this study, the impact of honey bee colony strength in the spring inspection on the colony strength at the time of pollination, the amount of foraged pollen and on the colony strength in autumn was observed. The honey bee colonies were, after the spring inspection, divided into two groups, based on the amount of bees. The weak colonies, in spring inspection, had an average up to 4 frames occupied by bees and the strong colonies, in the spring inspection, had more than 6 frames occupied by bees. In addition to the amount of bees, the amount of brood and food supplies were assessed in the inspections. It was determined that the stronger colonies had more pollen foragers in all three year of observation. The quantity of foraged pollen, in addition to strength in the spring inspection, was influenced by year. In two years (first and third) more pollen and larger quantity of red clover pollen was collected by the strong colonies, while in the second year, more pollen and a large quantity of red clover pollen was collected by weak colonies. In the fall inspections was found that the strong colonies still had more bees and brood, more pollen and, also, more honey in relation to the weak colonies.

Keywords: honey bee, red clover, colony strength, amount of foraged pollen

Introduction

Unlike alfalfa, red clover is almost entirely cross-pollinated species (97-98%), and selfsterility is caused by gamete incompatibility. Flower morphology of this crop allows entomophile pollination, and the most significant pollinators are honey bee (*Apis mellifera* L.), bumble bees (*Bombus* spp.) solitary bees and others, (Taylor and Smith, 1979). A number of authors prefer bumblebees, but there are those that give primary role to honey bee (*Palmer-Jones, 1967; Jevtić et al, 2010*

and others). *Rao and Stephen (2009)* have found that in the isolation cages with bumble bees 661 kg ha^{-1} of red clover seed was obtained and 640 kg ha^{-1} in cages with honey bees of red. In free fertilization without isolation, the yield was 1127 kg ha^{-1} seed.

Even that from spring inspection (late March) to early flowering of red clover (early July) three months pass, the strength of the colonies after the winter is the surest indicator of how they will behave in given year and what will be their role in pollination. Carnica overwinter in much weaker colonies than other races of honey bees. However, although it overwinters with significantly less bees than *A. m. ligustica*, carnica develops rapidly in spring and is very productive when it comes to honey foraging (*Kulinčević 2006*). In conditions of our country, very little was done in the issue of red clover pollination, so there are no results on the impact of honey bees in the pollination of this crop, let alone the necessary condition of bee colonies in order to perform this operation more successfully.

The aim of this study was to determine how much the colony strength established in the spring inspection has an impact on the development of colonies during the year and how much it influences the foraging activity of colonies in the summer at the time of pollination of forage crops, especially red clover.

Material and methods

In this study 20 honey bee colonies were used, hives were LR type with ten frames. Honey bees used in this experiment were of local ecotype of *A. m. carnica* Poll race. The trial lasted three years. Queen bees were 1-2 years old, and no colonies had queen bee older than 2 years. All included colonies were treated with same api-technical measures (all were feed the same, had the same stimulation of development). Colonies were treated against *Varroa* mite twice, in August (piretroids) and November (oxalic acid). Colonies wintered in the courtyard of the Institute for forage crops, and moved to the red clover field near Ćuprija. In the first year, colonies were located in the red clover field of 16 ha. In the second and third years, the colonies were at the same site, but the field was somewhat smaller (10 ha). In all three years the second cut was left for seeds production. Spring inspection was carried out in late March, and the colony strength and food supply were determined. The colony strength was determined visually through the area of frames occupied by bees (1/10) and the area under the brood, by method from Rulebook on the performance testing in breeding livestock (*S.G. R.S., 1996*). Based on the strength determined in the spring inspection, colonies were divided into two groups. The first group (poor colonies) was comprised of colonies that had approximately 4 frames with bees and 1.5 frames with brood frames. In the second group (strong colonies) were ranked colonies that had an average of 6.5 frames with bees and 2.5 frames with brood. The amount of honey and pollen was determined visually, similar as two previous traits. Colonies were brought in the

crop field in early July, when the clover started to flower (10-15% flowers bloomed) and remained there for about three weeks. During red clover flowering, the counting of scout and forager bees was performed 5 times and pollen was taken using collectors. The number of bees was determined by counting scouts and foragers (bees with a load of pollen) which returned to the hive for 3 minutes. The total amount of pollen per colony was determined by using the pollen collectors to seize the pollen from forager bees which entered the hive. The obtained pollen was dried and measured in order to determine the total amount of collected pollen. After extracting the red clover pollen (based on color and microscopic analysis), its quantity was measured. Upon the end of red clover flowering, colonies were returned to the stationary apiary in Kruševac, where they wintered. The fall inspection was carried out in the first ten days of September, and the same parameters were observed by the same methodology that was used during the spring inspection.

Results and discussion

The colonies, in the spring inspection, had an average of 5.2 frames occupied with bees, weak had 3.9 frames, and strong had 6.6 (Table 1).

Table 1. Spring inspection of honey bee colonies, 2005-2007

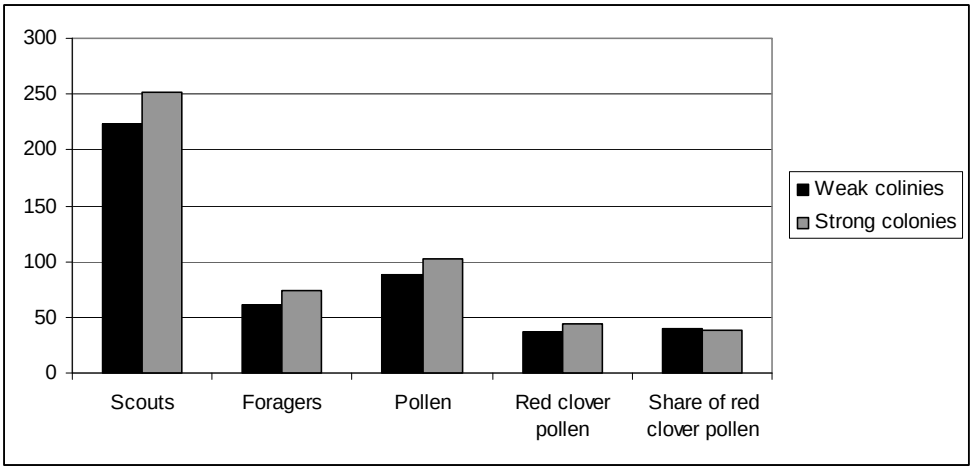
	Bees	Brood	Honey	Pollen
Weak colonies				
2005.	3.92	1.65	3.56	0.74
2006.	3.78	1.52	3.22	0.53
2007.	4.08	2.11	3.55	0.68
Average	3.93	1.76	3.44	0.65
Stand. dev.	0.60	0.44	0.75	0.26
CV	15.62	27.60	21.79	39.04
Strong colonies				
2005.	6.53	2.61	3.31	0.82
2006.	6.42	2.80	3.72	1.02
2007.	6.78	2.75	3.81	0.98
Average	6.58	2.72	3.61	0.94
Stand. dev.	0.80	0.54	1.37	0.43
CV	12.43	20.37	39.71	48.88
Total average	5.225	2.240	3.525	0.800

The amount of brood varied more than amount of bees, especially in weaker colonies in the second year. In contrast to the colony strength, the food supply was much more uniform, especially regarding the amount of honey in the spring inspection. The colonies had an average of 3.5 frames of honey, and the differences between the groups were very small. In the first year weak colonies had

more honey, and in the second and third years the strong colonies. There was a clear differentiation between the colonies when it comes to the amount of pollen. In all three years, strong colonies had more pollen in the spring inspection. In particular, the second year must be noted when the strong colonies had double the amount of pollen. In addition to the differences between the groups, the high intra-group variation for observed traits was determined. The variation coefficient (CV) for the amount of pollen had the highest value in relation to all observed traits and was high for the amount of pollen in weak colonies and the amount of honey in strong colonies (Table 1.).

The colony strength in the spring inspection depends on many factors, but primarily on climatic conditions, colony strength in wintering and measures taken in the spring. *Nedić et al. (2011)* found that the colonies of four selected lines had an average of 2.8 frames with bees, 3.9 frames with brood, 2.5 frames with honey and 0.5 frames with pollen in spring inspection. In the research of *Jevtić et al. (2012)* colonies in the spring inspection had an average of 5 frames with bees, 1.9 frames with brood, 3.6 frames with honey and 0.6 frames with pollen. *Jevtić et al. (2004)* concluded that the colony strength is affected by the amount of solid food that was feed to colonies during the winter. In addition, it was found that the strong colonies best wintered, had no loss during winter and in both observed years maintained superiority for the amount of bees and brood in relation to weak colonies (*Jevtić et al., 2005*).

When it comes to red clover pollination, there were high differences both in the colony strength and in the amount of foraged pollen (Graph 1.). The strong colonies, at the time of red clover pollination, had 13% more scouts (251 scouts) than weak colonies (222 scouts). In addition, the strong colonies had 20.7% more foragers (74 in strong colonies, 61 in weak). During the study, the colonies foraged an average 94.9 g of pollen, strong colonies collected 102.2 g and weak 87.6 g. The difference of 14.6 g in benefit of strong colonies shows that they foraged 16.7% more pollen. Strong colonies, in addition to the total amount of pollen, foraged larger quantity of red clover pollen in relation to weak colonies. Exception is in the second year when weak colonies foraged larger amount of both the total pollen (61.46 g) and red clover pollen (16.06 g) compared to the strong colonies (46.7 g of total pollen and 9.9 g of red clover pollen). For these two traits there is a large intra-group variation.



Graph 1. Average number of scouts and foragers, amount of foraged pollen, amount of foraged red clover pollen and share of red clover pollen 2005-2007

Maxfield-Taylor and Rao (2011) found, in two-year study, that honey bees, in both the early (July) and medium period (end of July, beginning of August), of the total amount of foraged pollen, foraged mostly pollen of red clover. In the late period (mid-August), in the first year, there was no red clover pollen, while in the second year red pollen was again dominant in the total amount of collected pollen (92%).

Table 2. Number of scouts and foragers, amount of foraged pollen and amount of foraged red clover pollen 2005-2007

	scouts	foragers	pollen	Red clover pollen	Red clover pollen share (%)
Weak colonies					
2005.	247.32	54.23	109.15	56.43	51.69
2006.	195.84	64.48	61.22	16.06	26.23
2007	224.73	65.58	92.43	38.42	41.56
Average	222.63	61.43	87.60	36.97	39.83
Stand. dev.	42.80	19.06	48.59	26.59	15.18
CV	19.57	26.21	49.24	73.19	46.48
Strong colonies					
2005.	288.53	69.33	147.30	77.07	54.47
2006.	218.94	74.92	46.74	9.91	17.48
2007.	247.43	78.24	112.58	46.48	41.29
Average	251.63	74.16	102.21	44.49	37.75
Stand. dev.	72.76	9.32	52.31	34.69	19.77
CV	33.07	14.84	61.95	98.87	63.06
Mean average	237.130	67.797	94.903	40.730	38.790

After completion of pollination, the colonies were prepared for the winter. There were still differences in the strength and food supplies in fall inspection between the colony groups that were determined in the spring inspection (Table 3). Strong colonies had more bees (9.3 frames) in fall inspections, in relation to medium strong colonies (7.5 frames). Brood area was identical in both groups. Strong colonies had 1.3 frames more of honey which is about 3 kg or 27.4% more. Strong colonies had more pollen. It is particularly interesting to note a high variation coefficient for this trait in both groups of colonies. This leads to the conclusion that in the group of strong colonies there were those who had had low supply of pollen, but also those in group of weak colonies with slightly more pollen. In the research of *Nedić et al. (2011)*, colonies in fall inspection had approximately 5.3-6.1 frames with bees and average 5.14 frames with brood. In ten-year observation by *Jevtić et al. (2012)*, it was found that the colonies, in fall, had an average of 6.1 frames with bees, 1.1 frames with brood, 4.4 frames with honey and 0.3 frames with pollen.

Table 3. Fall inspection of honey bee colonies, 2005-2007

	Bees	Brood	Honey	Pollen
Weak colonies				
2005.	8.08	2.10	4.64	0.34
2006.	6.96	1.58	4.86	0.16
2007.	8.18	1.45	4.98	0.42
Average	7.74	1.71	4.83	0.31
Stand. dev.	1.42	0.96	1.48	0.24
CV	18.46	49.76	31.41	86.70
Strong colonies				
2005.	9.28	1.78	5.57	0.38
2006.	9.30	1.86	6.44	0.40
2007.	9.89	1.69	6.58	0.48
Average	9.49	1.78	6.20	0.42
Stand. dev.	2.54	1.07	1.48	0.27
CV	27.34	59.47	25.26	70.28
Total average	8.615	1.745	5.515	0.365

Conclusion

After three-year observation, honey bee colonies in spring inspections had average of 5.2 frames with bees, 2.4 frames with brood, 3.5 frames with honey and 0.8 frames with pollen. Colonies that have been strong in the spring inspection showed the greater strength during red clover pollination due to higher number of scout bees and forager bees. Strong colonies were significantly more effective at the time of red clover pollination, as they collected for 16.8% more total pollen and red

clover pollen for over 20.3% more. There is exception in the second year of observation when weak colonies foraged more total pollen and red clover pollen. Weak colonies foraged less red clover pollen compared to the strong colonies, but the proportion of red clover pollen in the total amount of collected pollen was slightly higher among them (39.8%) than in strong colonies (37.7%). In fall inspection strong colonies have maintained their superiority over the weak colonies because they had more bees and greater food supplies, while the amount of brood in this survey was quite uniform.

Acknowledgment

This research has been funded by Ministry of education and science of Republic of Serbia (Project TR31057).

Snaga pčelinjih društva na prolećnom pregledu i njen uticaj na količinu sakupljanja polena u vreme oprašivanja crvene deteline

G. Jevtić, B. Anđelković, Z. Lugić, N. Nedić, K. Matović

Rezime

U radu je praćeno kako, snaga društava utvrđena na prolećnom pregledu, utiče na snagu društava u vreme oprašivanja crvene deteline, na količinu sakupljenog polena, količinu polena crvene deteline i na snagu društva na jesenjem pregledu. Društva su nakon prolećnog pregleda, a na osnovu količine pčela, svrstana u dve grupe, od po 10 društava. Slaba društva su na prolećnom pregledu imala prosečno do 4 rama zaposednuta pčelama, a jaka društva su prosečno na prolećnom pregledu imala više od 6 ramova sa pčelama. Pored količine pčela na pregledima je praćena i količina legla, i zaliha hrane. Praćenje je trajalo tri godine, utvrđeno je da jaka društva imaju više izletnica i više polenarica u vreme oprašivanja crvene deteline u sve tri godine posmatranja. Na količinu sakupljenog polena pored snage društva na prolećnom pregledu uticaj ima i godina. U dve godine (prvoj i trećoj) više polena ukupno i veću količinu polena crvene deteline sakupila su jaka društva, dok su u drugoj godini nešto više polena ukupno i veću količinu polena crvene deteline sakupila slaba drustva. Na jesenjem pregledu je ustanovljeno da su jaka društva i dalje imala veću snagu (više pčela i legla), više polena, ali i više meda u odnosu na slaba društva.

References

JEVTIĆ G., MLADENOVIĆ M., NEDIĆ N., DINIĆ B. (2004): Uticaj količine čvrste hrane na zimovanje pčelinjih društava. Biotechnology in Animal Husbandry, Vol.20, N^{o} 5-6, str. 363-368.

JEVTIĆ G., MLADENOVIĆ M., NEDIĆ N. (2005): The Influence of the Quantity of Honeybees and Honey Reserves on Wintering of Honeybee Colonies. 8th International Symposium Modern Trends In Livestock Production Belgrade Zemun, Serbia and Montenegro 5 – 8th October. 315-321.
JEVTIĆ G., ANĐELKOVIĆ B., LUGIĆ Z., MLADENOVIĆ M., NEDIĆ N. (2010): The influence of the hive distance and the use of corn syrup on pollinator visits and red clover seed yield. Proceedings of the XII International Symposium on Forage Crops Of Republic of Serbia. Kruševac-Serbia 26-28 May, Book 2, 167-172.
JEVTIĆ G., ANĐELKOVIĆ B., SOKOLOVIĆ D., ANĐELKOVIĆ S., MLADENOVIĆ M., NEDIĆ N., SIMEONOVA V. (2012): Impact of flowering time of major honey plants on honey bee colony development and honey yield in Rasina region. The first international symposium on animal science, Book II, Faculty of Agriculture, Institute for Zootechnique, Novembar 8-10th, Belgrade, Serbia, 957-965
KULINČEVIĆ J. (2006): Pčelarstvo. Četvrto dopunjeno izdanje. Izd. Partenon Beograd. 1-322.
MAXFILD-TAYLORS., RAO S. (2011): Characterization of pollen loads from pollen traps placed in honey bee hives in red clover seed fields in the willamette valley http://cropandsoil.oregonstate.edu/seed-ext/sites/default/files/2-SR-11-06-Maxfield-Taylor-Characterization-of-Pollen-Loads.pdf
NEDIĆ N., STOJANOVIĆ Z., JEVTIĆ G., PLAVŠA N., MATOVIĆ K. (2011): Variability of production characteristics of distinguished lines of bees in Western Serbia. Proceedings 3rd International Congress "New Perspectives and Challenges of Sustainable Livestock Production" Belgrade, Republic of Serbia 5 – 7th October. 1379-1386.
NEDIĆ N., MALETIĆ R., MARKOVIĆ M., JEVTIĆ G., ANĐELKOVIĆ B., MATOVIĆ K. (2012): Morphological differentiation of honeybees (*Apis mellifera*) from Serbia. The first international symposium on animal science, Book II, Faculty of Agriculture, Institute for Zootechnique, Novembar 8-10th, Belgrade, Serbia, 948-957.
PALMER-JONES (1967): Honey bees as pollinators of red clover. New Zeal. Jour. Agr. 114: 34-35.
RAO S., STEPHEN W. P. 2009. Bumble Bee Pollinators in Red Clover Seed Production. Crop Science, 49: 2207-2214.
SLUŽBENI GLASNIK R.S. br. 21, 16.05. (1996): Pravilnik o načinu ispitivanja svojstava priplodne stoke i o uslovima proizvodnje i transporta živine.
TAYLOR N.L., SMITH R.R. (1980): Red Clover Breeding and Genetics. Advances in Agronomy, 31, 125-154.

Received 26 December 2012; accepted for publication 15 February 2012

Biotechnology in Animal Husbandry 29 (1), p 123-132 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 633.3
DOI: 10.2298/BAH1301123C

INFLUENCE OF SOME BIO-PRODUCTS ON THE BIOLOGICAL AND PRODUCTIVE CHARACTERISTICS OF BIRD'S FOOT TREFOIL GROWN FOR FORAGE

B. Churkova

Institute of Mountain Stockbreeding and Agriculture - Troyan
Corresponding author: bchurkova@abv.bg
Original scientific paper

Abstract: During the 2010-2012 period the influence of some bio-products being mainly a combination of macro and micronutrients at different concentrations on the productivity, botanical and morphological composition of bird's foot trefoil swards was studied. The experiment was carried out on the experimental field of the IMSA – Troyan by the completely randomized method with 4 replications and harvest plot size of 5 m^2. Four bio-products phosphorus humate in dose of 3000 l/ha, boron humate (1600 l/ha) and molybdenum humate (1600 l/ha) and their combination phosphorus humate (2500 l/ha) + boron humate (1000 l/ha) + molybdenum humate (1000 l/ha) were studied. They were applied at the 2-4 leaf. The results showed that solely phosphorus humate had a positive influence, which increased the dry matter yield only by 8.7% and the results were not statistically significant.. It was found that the data had a unidirectional character with regard to the leafiness degree during the years as a result of the applied bio-fertilizers. Their application had a positive effect on the leafiness. The phosphorus humate showed a tendency to increase the relative portion of stems in the sward from the first to the third year. The kind of the applied bio-fertilizers was not of substantial importance to the degree of stem growth and botanical composition of the sward.

Key words: bird's foot trefoil, bio-products, productivity, botanical and morphological composition.

Introduction

During recent years more and more attention has been paid to the search of alternative, ecologically friendly solutions for maintenance of the nutrient regime, which should correspond to the contemporary farming *(Kephart et al.,*

1995; Goranova, 2007; Liebman and Davis, 2009) One of the prerequisites for obtaining of high-productive and good-quality forage from bird's foot trefoil is to provide a suitable nutrient environment with optimal conditions for plant development *(Churkova and Lingorski, 2010).* Lately more and more attention has been paid to fertilizing by application of bio-products *(Kephart et al., 1995)*, which contain small quantities of nutrients. The bio-fertilizers are a solution in conformity with nature which can improve nutrient regime of plants and increase their productivity*(Vanotti et al., 1997).* The series of Organic mineral fertilizers has been made with different proportions of nutrients according to the specific requirements of the agricultural crops by developmental stages. The micronutrients are in a chelated form for maximum availability.

The obtaining of a positive effect on the formation of forage yield conditions the necessity for a continuous influx of nutrients *(Nikolova, 2009).* When expecting a higher yield the recommendation is for a starting foliar fertilizing at the stage of early vegetation with Nitrohumate at the dose of 3000 ml/ha and a supplementary fertilizing – after each cut with Nitrohumate and Phosphorus humate *(www.agrobiostim.com)* at the dose of 3000-3500 ml/ha with an working solution of 300-400 liters per hectare, aiming at fast growth of the plants and increase of the yield from the next cuts. In case of symptoms of potassium deficiency it is recommended to treat with potassium humate at the dose of 3000 ml/ha. The supplementary fertilizing with bio-fertilizers containing the main nutrients at the suitable ratio resulted in: an increase of the green mass yields of more than 80-100%, improvement of the produce quality and neutralization of the influence of herbicide residues in soil *(Watson et al., 2002).*

For normal functioning of the root nodules and when growing bird's foot trefoil on grey forest soils it is necessary to treat the stands with a natural microfertilizer, such as boron and molybdenum humate.

The supplementary fertilizing of stands with the organic fertilizers supplies the plants with all necessary macro and micronutrients, because the bio-fertilizers have high content of zinc and manganese and readily available forms of boron and molybdenum.

In many countries these bio-preparations are at a stage of profound research. In Bulgaria the studies on their application to the forage crops are not sufficient.

The objective of this study was to investigate the influence of foliar treatment with different bio-fertilizers applied alone and combined on the growth and development, productivity, botanical and morphological composition of bird's foot trefoil swards.

Material and methods

The trial was carried out during the 2010-2012 period in the experimental field of IMSA - Troyan by completely randomized method with 4 replications and harvest plot size of 5 m^2 with bird's foot trefoil variety Targovishte 1. The following bio-fertilizers were tested: Phosphorus humate, Boron humate and Molybdenum humate. All preparations are products of the manufacturer company Agro Bio Stim – Bulgaria. The biofertilizer application was conducted with an working solution of 3000 liters per hectare at the 2-4 leaf stage of bird's foot trefoil in the first year and at the beginning of vegetation in every next year. The following variants were studied: control – zero, phosphorus humate (3000 l/ha), boron humate (1600 l/ha) and molybdenum humate (1600 l/ha) and phosphorus humate (2500 l/ha) + boron humate (1000 l/ha) + molybdenum humate (1000 l/ha). The treatment with the mentioned doses was in conformity with the quantities according to the recommendations of the manufacturer company. A generally adopted technology for growing of bird's foot trefoil for forage was applied. The sward sowing was conducted by hand, broadcast, at the sowing rate of 0.12 tha^{-1}.

The phosphorus humate is a combination of nitrogen, phosphorus and potassium in the composition of 4-12-4%, magnesium - 0.5% and the micronutrients: calcium, boron, iron, manganese, cobalt, zinc and molybdenum, as well as the organic substances: humic acids and fulvic acids. The boron humate composition is the following: total nitrogen - 6%, boron - 5%, organic carbon - 0.4% and the organic substances: humic acids, fulvic acids, amino acids: valine, glutamine, methionine, lysine, antibiotics, vitamins, micronutrients – chelated iron. The molybdenum humate composition includes as follows: natural stimulants + nitrogen – 6%, molybdenum – 9%, organic carbon – 0.4%, humic acids, fulvic acids, amino acids: valine, glutamine, methionine, lysine, antibiotics, vitamins.

The following characteristics were recorded: dry matter yield (tha^{-1}) determined by cuts and years by drying of average samples to constant weight at 105 C^0. botanical composition of the sward determined just before harvesting of the first cut in a weight percentage (%) through taking of average samples from each replication; morphological composition – determined by weighing an average sample of stems, leaves and generative organs from each variant and each replication; plant height (cm) – measured at the stage of budding-early flowering of 40 plants from each variant taken from each replication. We conducted the sward harvesting at the stage of budding-early flowering.

Mathematical processing of the primary data on the studied characteristics was performed according to *Lidanski* (*1988*).

Results and discussion

The fertilizing effect is connected to a great extent with the climatic conditions. The year 2010 was considered favourable with regard to the rainfalls, when these were distributed evenly in the months of the growing season. The average daily air temperature of 10.8 C^0 and rainfall quantity of 112.6 l/m^2 in April had a favourable effect on the normal emergence of bird's foot trefoil. The 2-4 leaf phenological stage occurred normally in mid-April, and the budding in mid-June. That contributed to formation of a comparatively good first cut, being harvested in late June, when the stage of budding-early flowering occurred. The high soil moisture due to the rainfall quantity of the range of 76.7 and 132.7 l/m^2 ensured fast growth and development of bird's foot trefoil after its mowing and formation of a second cut of 33-day duration.

In the second year there was a considerably smaller quantity of rainfalls in the months of March (41.7 l/m^2); April (68.0 l/m^2) and May (69.1 l/m^2) in comparison with the months of June (98.4 l/m^2), July (72.9 l/m^2) and August (96.8 l/m^2). That did not influence the sward productivity, and as a result, high productivity of the sward was recorded in all variants after the biofertilizer application.

The agro-meteorological characteristics of the third year differed very much from the other two years. The uneven rainfall distribution by months was very pronounced, characterized by a good water supply in May (174.1 l/m^2) and a lack of rainfall in July. The drought affected the later summer months, when the second cut had been already harvested. The good supply of soil in the first months of the growing season contributed to obtaining of a good and stable yield from the first cut, which in combination with the high drought resistance of bird's foot trefoil, necessary for formation of a second cut, ensured a good yield for the year.

In the first year, the individual treatment of bird's foot trefoil with Phosphorus humate and Molybdenum humate showed a higher dry matter yield (Table 1) than that of the control. At doses of Phosphorus humate of 3000 ml/ha and Molybdenum humate of 1600 ml/ha the exceeding at the sward harvesting was by 8.0 and 3.8%. The bird's foot trefoil treatment with boron humate and the combination of the three bio-products decreased the fertilizing effect and the yields obtained for these treatments were lower than the control

Table 1. Dry mass yield (tha^{-1}) by years and on average for the period

Variants	2010		2011		2012		On average for the period	
	tha^{-1}	%	tha^{-1}	%	tha^{-1}	%	t.ha^{-1}	%
Control - untreated	38.9 -	100.0	116.8	100.0	82.1-	100.0	79.3	100.0
Phosphorus humate	42.0-	108.0	123.9-	106.0	92.5-	112.7	86.1-	108.7
Boron humate	32.6-	83.8	106.9-	91.5	79.2-	96.5	72.9-	92.0
Molybdenum humate	40.4-	103.8	112.1-	96.0	73.4-	89.4	75.3-	95.0
Phosphorus humate + Boron humate + Molybdenum humate	38.3-	98.3	116.2-	99.4	77.3-	94.1	72.2-	97.4
GD 5%	16.9	43.5	18.9	16.25	15.5	19.0	16.0	20.3
GD1%	23.7	61.0	26.6	22.8	21.8	26.6	22.5	28.4
GD 0,1%	33.5	86.1	37.6	32.2	30.8	37.6	31.8	40.1

In the second year, the highest dry matter yield was recorded for bird's foot trefoil treated with Phosphorus humate (123.9 tha^{-1}), what exceeded over the control 6.0%. All other doses and kinds of bio-fertilizers showed negative effect on the bird's foot trefoil productivity. The lowest effect on the productivity was recorded for the treatment with Molybdenum humate (106.9 tha^{-1}), which was 8.5% lower than the control.

In the last year, the highest dry matter yield of 92.6 tha^{-1} was recorded for the treatment with Phosphorus humate what was 12.7% over the control. The high productivity in this variant was due to the role of the organic mineral fertilizer Phosphorus humate to increase the plant resistance to low temperatures and drought, which confirmed the characteristics of this bio-fertilizer provided by the its producer. When treating bird's foot trefoil with boron and molybdenum humate and the combination of the three bio-fertilizers, the effect was reduction of the yield, but it was not statistically significant. The variant treated with Molybdenum humate had the lowest productivity and as a result the dry mass yield was 73.4 tha^{-1}, which was 10.6% lower than the control and as a result of this was determined statistical significant difference between phosphorous humate and Molybdenum humate, at level 005.

When examining the bird's foot trefoil treatment with the different bio-fertilizers and the applied combination during the three years, it is noticeable that Phosphorus humate alsvaus gave positive effects on yield. That was due to stimulation of the growth and development of the root system because of the easy assimilability of the bio-product.

On average for the period of study the productivity for the treatment with Phosphorus humate alone at the dose of 3000 ml/ha was the most efficient, and as a result the productivity exceeded the control by 8.7%. There was not determined significant difference between the treatment and control. The productivity in all other variants was lower, which was of importance to differentiated use of the bio-

preparations and their careful application as a stage of the bird's foot trefoil technology.

In the first year, an average value of leafiness of X=44.6% was recorded (Table 2), the maximum being in the sward treated with Boron humate – 46.3 %. In the second year, the combination of Phosphorus humate + Boron humate + Molybdenum humate proved to be the most efficient with regard to this character. In 2012 the plants treated with Phosphorus humate had a leafiness percentage of 45.1%, at an average value of X=42.9%. During all years a low degree of variability for this character was recorded. The kind of the bio-products applied, had no influence on the stem quantity in the first year. In all variants they were more than those in the control variant, but in the treated variants they were 39.3 to 43.6%.

Table 2. Morphological analysis (%) by years for first cut

Variants	Leaves			Stems			Generative organs		
	2010	2011	2012	2010	2011	2012	2010	2011	2012
Control - untreated	43.2	39.4	41.7	29.1	49.4	45.6	27.7	11.2	12.7
Phosphorus humate	43.6	38.0	45.1	43.6	54.0	50.5	12.7	8.1	4.4
Boron humate	46.3	43.7	41.2	42.3	48.7	47.1	11.3	7.6	11.6
Molybdenum humate	46.4	40.3	36.7	39.3	52.8	45.7	14.3	6.8	7.6
Phosphorus humate + Boron humate + Molybdenum humate	43.6	44.0	39.8	41.3	49.2	48.7	15.2	6.8	11.5
X	44.6	41.1	42.9	39.1	50.8	47.5	16.2	8.1	9.6
SD	1.6	2.7	2.9	5.8	2.4	2.1	6.6	1.8	3.5
VC	3.4	6.5	6.7	14.9	4.7	4.4	40.5	22.5	36.3
Min	43.2	38.0	39.8	29.1	48.7	45.6	11.3	6.8	4.4
Max	46.4	44.0	46.7	43.6	54.0	50.5	27.7	11.2	12.7

The stem quantity in the second year considerably exceeded their quantity in the first year. During the three years they were in the maximum quantity in the sward treated with Phosphorus humate (43.6; 54.0; 50.5%). In the first year the variation coefficient was the highest CV=14.9, and the degree of variability was medium. According to the variation coefficients in the second and third year (CV=4.7 and 4.4%), the degree of variability for this character was very low. The generative organs during the three years were in the maximum quantity in the control variant - 27.7; 11.2 and 12.7%, and the degree of variability was very high.

In the first year of the experimental period, the highest values of the stem height (Table 3) were found for the plants treated with Phosphorus humate - 31.7 cm. The average value of stem height was 29.7 cm. In the second year, the bird's foot trefoil stems were considerably taller than those in the first year for the

variants treated with all kinds of bio-products, those treated with Molybdenum humate having a value of 49.8 cm. In the third year, the heights had values almost similar to those in the second year. They varied from 43.3 to 45.4 cm, at an average value of 44.4 cm. On average for the period of study, all treated variants showed higher growth, as compared to the control. The kind of the applied bio-fertilizers was not of substantial importance to the degree of stem growth. That was evident from the difference in the values between the treated variants, being 40.0 to 41.7 cm. The degree of variability of the height character, according to the variation coefficient, was very low by years, as well as on average for the period – VC=7.0; 6.8; 2.0 and 4.5%.

Table 3. Stem height (cm) by years and on average for the period

Variants	2010	2011	2012	On average for the period
Control - untreated	26.1	41.2	43.3	36.9
Phosphorus humate	31.7	46.8	43.8	40.8
Boron humate	30.1	45.4	45.4	40.3
Molybdenum humate	30.4	49.8	44.9	41.7
Phosphorus humate + Boron humate + Molybdenum humate	30.2	44.8	45.0	40.0
X	29.7	45.6	44.5	39.9
SD	2.1	3.1	0.9	1.8
VC	7.0	6.8	2.0	4.5
Min	26.2	41.2	43.3	36.9
Max	31.7	49.8	45.4	41.7

The effect of fertilizing with bio-fertilizers was exceptionally favourable on bird's foot trefoil at all doses and kinds with regard to the botanical composition of the sward (Table 4). The bird's foot trefoil participation in all variants exceeded that of the control. In first cut the highest portion of bird's foot trefoil was recorded when applying boron humate (95.8%). Among the treated variants, the degree of weed infestation was the highest for the bird's foot trefoil treatment with Molybdenum humate (16.3%).

In the second year, the fertilizing with bio-fertilizers proved to be very efficient with regard to the bird's foot trefoil participation. Bio-fertilizers had no positive influence on the botanical composition of the sward. In this year a positive tendency was retained towards a decreased degree of weed infestation after the application of all bio-fertilizers. That was connected to a great extent with the bird's foot trefoil biology and its maximum rate of growth and development in the second year of the experimental period. The difference between the variants in the weed infestation degree was insignificant being by variants: 0.3%; 0.4%; 0.3% and 1.3%, respectively.

Table 4. Botanical composition of the sward by years for first cut (%)

Variants	2010		2011		2012	
	Bird's foot trefoil	Weeds	Bird's foot trefoil	Weeds	Bird's foot trefoil	Weeds
Control - untreated	80.0	20.0	98.9	1.1	84.5	15.5
Phosphorus humate	93.3	6.7	99.7	0.3	88.7	11.3
Boron humate	95.8	4.2	99.6	0.4	80.9	19.1
Molybdenum humate	83.7	16.3	99.7	0.3	89.8	10.2
Phosphorus humate + Boron humate + Molybdenum humate	90.4	9.6	98.7	1.3	82.4	17.6

In the third year of the experimental period in first cut, the highest relative portion of bird's foot trefoil in the sward was found for its treatment with molybdenum humate, and as a result its participation was 89.2%. The sward treated with Phosphorus humate also had a low degree of weed infestation– 11.3% and a comparatively high presence of bird's foot trefoil, 88.7%.

Conclusion

Among the studied bio-products, the organic mineral fertilizer phosphorus humate had the strongest effect and when applying it to a pure sward in the first year at the 2-4 leaf stage, and in the next years at the beginning of vegetation it increased the dry matter yield and could be applied as an additional element of the technology for bird's foot trefoil. As a result of the applied bio-product at the dose of 3000 ml/da on average for the period of study the productivity exceeded the control by 8.7%, but there was not determined statistical significant difference.

It was found that the data had a unidirectional character with regard to the degree of leafiness during the years as a result of the applied bio-fertilizers. The phosphorus humate showed a tendency to increase the relative portion of the stems in the sward from the first to the third year.

The kind of the applied bio-fertilizers was not of substantial importance to the degree of stem growth and botanical composition of the sward.

Uticaj nekih bio-proizvoda na biološke i proizvodne osobine žutog zvezdana kao krmnog bilja

B. Churkova

Rezime

U periodu 2010-2012 ispitivan je uticaj nekih bio-proizvoda, uglavnom kombinacija mikro i makro elemenatau različitim koncentracijama na produktivnost, botanički i morfoloških sastav žutog zvezdana.Eksperiment je izveden na oglednom polju IMSA – Troyan, sa 4 ponavljanja i parcelama veličine 5 m^2.

Ispitivana su četiri bio-proizvoda fosfor humat u dozi od 3000 l/ha, bor humat (1600 l/ha) i molibden humat (1600 l ha) i njihova kombinacija fosfor humat (2500 l/ha) + bor humat (1000 l/ha) + molibden humat (1000 l/ha). Rezultati su pokazali da samo fosfor humat imapozitivan uticaj, što je povećalo prinos suve materije za samo 8,7%, a rezultati nisu bili statistički značajni.

Utvrđeno je da su podaci imala jednosmerna karakter u odnosu na stepena olistalosti tokom godina, kao rezultat primenjenih bio-đubriva. Njihova primena je imala pozitivan efekat na olistalost.Fosfor humat je pokazao tendenciju da poveća relativni deo stabljike u busenu od prve do treće godine.

Vrsta primenjenih bio-đubriva nije od suštinskog značaja za stepen raststabljike i botanički sastav.

References

CHURKOVA B. LINGORSKI V. (2010): Effect of leaf treatment with organic preparation alfalfa blend 5-5-5 on the forage yield and botanical composition of birds' foot trefoil. Journal of Mountain Agriculture on the Balkans, 13, 5, 1156-1164

GORANOVA. G. (2007): Importance of ecotypic selection for forage grass breeding, Journal of Balkan Ecology, 10, 2, 147-153.

KEPHART, K.D., WEST C.P., AND WEDIN D.A. (1995): *In* R.F Barnes et al. (ed.) Forages Volume I: An introduction to grassland agriculture. 5th edition. Iowa State Univ. Press, Ames, IA. Grassland ecology and improvement. p. 141-153.

LIDANSKI T. (1988): Statistical methods in biology and agriculture, Zemizdat, Sofia, 150-187

LIEBMAN M DAVIS A.S (2009): Managing weeds in organic farming systems: an ecological approach. In: Organic farming: The ecological system. Francis C, editor. Madison: American Society of Agronomy, 173–196.

Matter in Temperate Agroecosystems, CRC Press, Boca Raton, FL, pp 105-

NIKOLOVA M. (2009): Practical and economic problems of realization of the agri-ecologial activities in the field of horticulture. Scientific Research Almanac, Tsenov APH, Svishtov, 9, 43-79.

VANOTTI M.B., BUNDY L.G., PETERSON A.E. (1997): Nitrogen fertilizer and legumecereal rotation effects on soil productivity and organic matter dynamics in Wisconsin. In: Paul EA, Paustian K, Elliot ET Cole CV (Eds) Soil Organic
WATSON C.A., ATKINSON D., GOSLING P., JACKSON L.R., RAYNS F.W. (2002): Managing soil fertility in organic farming systems. Soil Use and Management, 18, 239-247

Received 3 January 2013; accepted for publication 19 February 2013

Biotechnology in Animal Husbandry 29 (1), p 133-143 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 637.07'05
DOI: 10.2298/BAH1301133C

SIVAS KOFTE AND EXAMINATION OF MICROBIOLOGICAL QUALITY

O. P.Can[1], S. Şahin[2], M. Erşan[3], F. Harun[4]

[1]Department of Food Engineering, Faculty of Engineering, University of Cumhuriyet, 58140 Sivas/Turkey.
[2]Department of Food Hygiene and Tecnology, Faculty of Veterinary Medicine, University of Cumhuriyet, 58140 Sivas/Turkey.
[3]Department of Chemical Engineering, Faculty of Engineering, University of Cumhuriyet, 58140 Sivas/Turkey.
[4]Institution of Agriculture and Rural Development, Erzincan/Turkey.
Corresponding author: ozlempelincan@gmail.com
Original scientific paper

Abstract: The objective of this study was to examine traditional meat product of the Sivas province, the Sivas kofte with regards to its microbiological quality. The kofte samples sold commercially were examined according to their microbiological qualities (150 pieces cooked kofte samples taken from the most popular 5 restaurants). The samples were analyzed in terms of total mesophilic aerobic bacteria, Enterobacteria, *E. coli*, coagulase positive *S. aureus*, *Salmonella* spp. and psychrophilic bacteria. Ready to serve samples of Sivas kofte were examined and the following results were obtained for total mesophilic aerobic bacteria, Enterobacteria, coagulase positive *S. aureus*, psychrophilic bacteria, 2.7-4.9 $\log_{10}$ cfu/g, <10 - 2.1 $\log_{10}$ cfu /g, <10 - 1.9 $\log_{10}$ cfu /g, 1.6- 3.8 $\log_{10}$ cfu /g, respectively. *E. coli* and *Salmonella* spp were not determined in any of the samples. As a result, the ready to consume Sivas kofte samples were found to be in accordance with the Turkish Food Codex Cominiquate Microbiological Criteria despite differences in the microbiological quality of the locations in Sivas.

Key words: Food hygiene, Microbiological quality, Sivas kofte.

Introduction

Nowadays, the changes in individual's consumption habits and advances in food technology have caused an increase in the demand for different style convenience and semi-processed convenience foods. Meatball is one of the most preferred foods due to the ease of preparation (*Anar, 2010*). The meat product prepared by using fresh mince and by shaping the meat dough, which is mostly consumed after grilling is known as a meatball (*TSE 1992*).

Meatballs prepared according to traditions of different regions (Inegol, Akcaabat, Sivas etc.) according to the content of meat dough has an important place in Turkish cuisine. Sivas kofte is one of these kinds and it is a meat product which has a geographical patent and is consumed by the local community. Sivas kofte which is a special product both in terms of its preparation and form of consumption is identified with the city of Sivas. Sivas kofte is produced in three phases of raw material, preparation and cooking. In order to get the special taste of the meatball the meat should be obtained from beef cattle or sheep which raised and bred in plateaus of the Sivas region and fed with clover, *vicia sativa* and marjoram. The rib, leg, and shoulder meat of beef cattle of these plateaus at the minimum age of two years along with leg of sheep are used as raw material. 20 g of salt is added for each kg of the mixture prepared and it is ground in mincing machine. No other material other than salt is used during the preparation of the mixture. The salt used is natural unprocessed salt produced in Tuzlagolu village, Zara district, Sivas. The ground meat mixture is left to rest for 12 hours. The meat is then again ground in the mincing machine with a moderate aperture. The mixture is sliced in to 25 g slices and an oval shape is given to the meat by hand. The meatballs prepared are grilled over an intense char coal fire by turning upside down in short intervals to cook both sides (*RG, 2010*).

Meatball is prepared by mince which is a quite suitable environment for microbial growth. The quality of mince and other additives used in preparation of meatballs determines the quality of meat products like meatballs (*Başkaya et al., 2004*). *E. coli*, *S. aureus*, *Cl. perfringens*, *B. cereus*, *Listeria* spp. and *Salmonella* spp. Together with other pathogens have negative effects on product quality and public health. The reasons of microbial growth in such food are high loads of microorganisms in food raw materials, inadequate thermal treatment, contaminant material, preservation in unsuitable environment, inadequate processing hygiene, cross contamination and unconscious personnel (*Gülmez et al., 2005*). Studies done on microbiological quality on ground meat show that ground meat is a good medium for the growth of microorganism like *S. aureus*, *Salmonella* etc (*Davidson et al., 2000*; *Philips et al., 2001*).

Since meatballs are sold as raw, they can spoil easily and contain some pathogens that pose threats to human health. The studies performed in Turkey showed that the microbial qualities of the meatballs are low and that some contain pathogen mechanisms (*Erol, 2007*).

This study was prepared to introduce Sivas kofte which is a traditional meat product of Sivas region, to determine its microbial quality and to protect public health.

Materials and Methods

The meatball samples examined in this study were taken from five restaurants selling the highest amount of meatballs in Sivas city centre. Sampling was done for 5 days in the restaurants and two groups of meatball samples were taken in each of the sampling days. A group of meatball sample is comprised of three meatballs. A total of 30 meatballs were sampled from each restaurant (6 pieces/day). Thus, a total of 150 grilled meat samples were sampled randomly just prior to the service. The samples were brought under cold chain and analyses were initiated on the same day.

10 g of meatball samples were taken into plastic bags under aseptic conditions and 90 ml % 0.1 water with peptone was added and stirred for 3 minutes after which serial distillations were prepared (ICMFS 1982).

Plate Count Agar (Oxoid CM325) medium was used for total mesophilic aerobic bacteria count. Petri plates were incubated at 35°C for 48-72 hours (*ICMFS, 1982*).

For Enterobacteria count Violet Red Bile Glucose Agar (VRB, Oxoid CM485) medium was used. Petri plates were incubated at 37±1 ºC for 18-24 hours (*ICMFS, 1982*).

25 g of sample was homogenized within 225 ml Maximum Recovery Diluent for *E.coli* determination. Hence, 10^{-1} dilution was prepared and was placed in a 0.5 ml Chromocult Tryptone Bile X-Glucuronide Medium (TBX) (CM945) which had been prepared before in accordance with the agar diffusion method. Then they were hold at 30±1°C for 4±1 hours and incubated at 44±1°C for 18±2 hours. After incubation blue-green coloured colonies in the medium were evaluated as *E. coli*. Since chromogenic medium was utilized confirmation was not done. As a positive control *E. coli* ATCC 25922 strain was used (*ICMFS, 1982*).

For psychrophilic bacteria counting Plate Count Agar (PCA, Oxoid CM325) medium was used. The plates were incubated at 7°C for 10 days (*ICMFS 1982*).

Coagulase positive *S. aureus* counting: Baird Parker (BP) agar (Oxoid CM275) was used for *Staphylococcus* counting. Petri plates were incubated at 37±1°C for 30 hours. After incubation the number of colonies which had a typical black coloured view surrounded by a light coloured area and atypical colonies were determined. Afterwards, five of these colonies were taken and coagulase test was employed. The number of the colonies having a positive coagulase test result was multiplied by suspicious colonies and divided into 5 so that the number of positive *S. aureus* was obtained (*ICMFS, 1982*).

For the isolation of *Salmonella* 25 g of sample was homogenized in 225 ml buffered water with pepton (T.P.S) during pre-enrichment stage and incubated at 37°C for 24 hours. During the selective enrichment stage, however, they were transferred into Rappaport Vassiliadis (R.V.) broth (Oxoid CM669) through 0.1 ml

TPS and incubated at 42°C for 24-48 hours. Then they were put into Brilliant Green Agar (B.G.A.) (Oxoid CM263) via circular loop and incubated at 37°C for 20-24 hours. The pink-reddish coloured colonies surrounded by bright red area in B.G.A. were evaluated as suspicious *Salmonella* spp. From these colonies some were put into Triple Sugar Iron Agar (T.S.I.A.) (Oxoid CM277) and Lysine Iron Agar (L.I.A.)(Oxoid, CM381) slant agar and incubated at 37°C for 24 hours. At the end of the incubation positivity evaluation of tubes was done according to the change in colour in T.S.I.A and L.I.A. Salmonella antiserum (Salmonella O Poly A-1 and Vi-Difco 2264-47-2) was used to test suspicious *Salmonella* spp. And the ones with positive agglutination formation were evaluated (*ICMFS, 1982*).

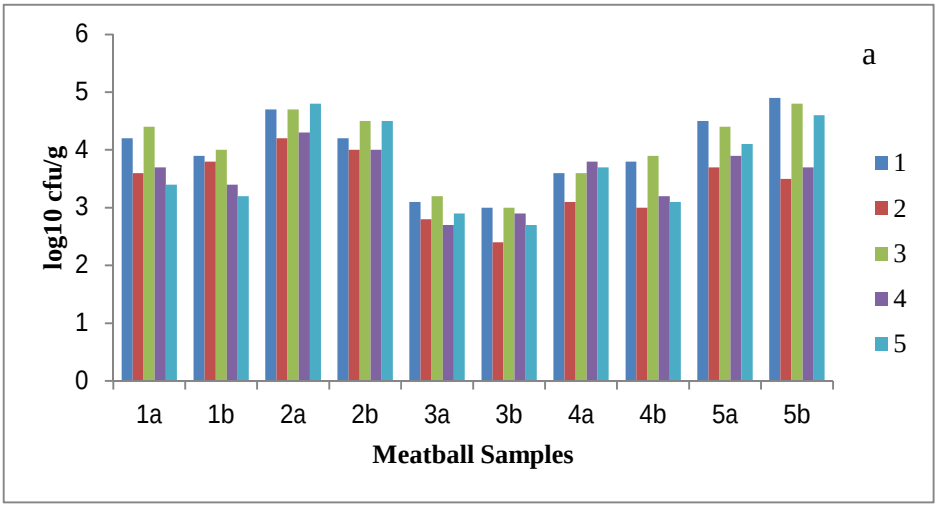
Results and Discussion

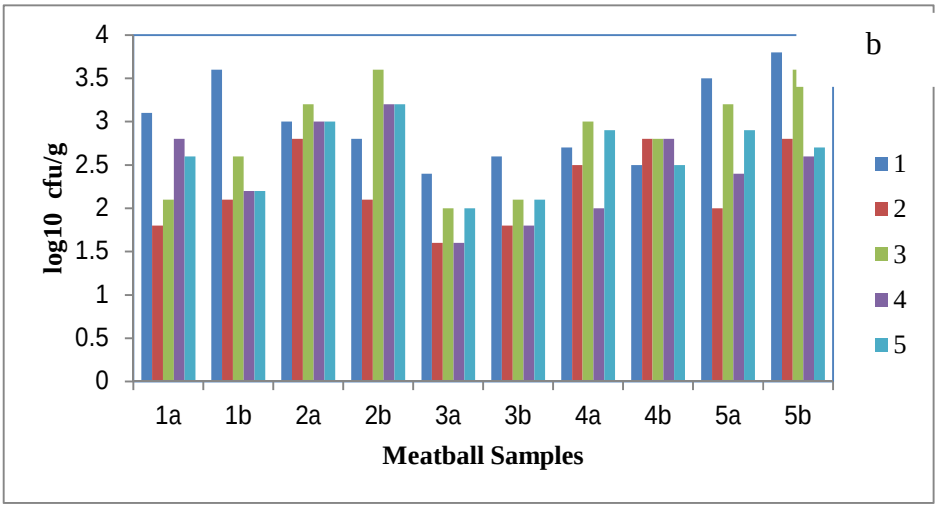
The number of total mesophilic aerobic bacteria, *Enterobacteria*, coagulase positive *S. aureus* and psychrophilic bacteria in 150 Sivas kofte obtained from five restaurants selling the highest amount of meatballs in Sivas city centre were found as 2.7-4.9 $\log_{10}$ cfu/g, <10 - 2.1 $\log_{10}$ cfu/g, <10 - 1.9 $\log_{10}$ cfu/g, and 1.6- 3.8 $\log_{10}$ cfu/g, respectively. No *E.coli* and *Salmonella* spp. Was determined in any of the meatball samples. The minimum and maximum values belonging to the meatball samples were given in Table 1. Microbiological analysis findings for the samples were given by Figure 1.

Table 1. The minimum and maximum values belonging to the meatball samples ($\log_{10}$ cfu/g)

Microorganism	No. of samples	Minimum	Maximum	No. of postive samples* (%)
Total mesophilic aerobic bacteria	150	2.7	4.9	150 (100)
Enterobacteria	150	<10	2.1	102 (68)
Coagulase positive *S. aureus*	150	<10	1.9	57 (38)
Psychrophilic bacteria	150	1.6	3.8	150 (100)

* The numbers determined over the determination limit are accepted as positive.


a
log10 cfu/g
0
1
2
3
4
5
6
1a
1b
2a
2b
3a
3b
4a
4b
5a
5b
Meatball Samples
1
2
3
4
5


b
log10 cfu/g
0
0.5
1
1.5
2
2.5
3
3.5
4
1a
1b
2a
2b
3a
3b
4a
4b
5a
5b
Meatball Samples
1
2
3
4
5

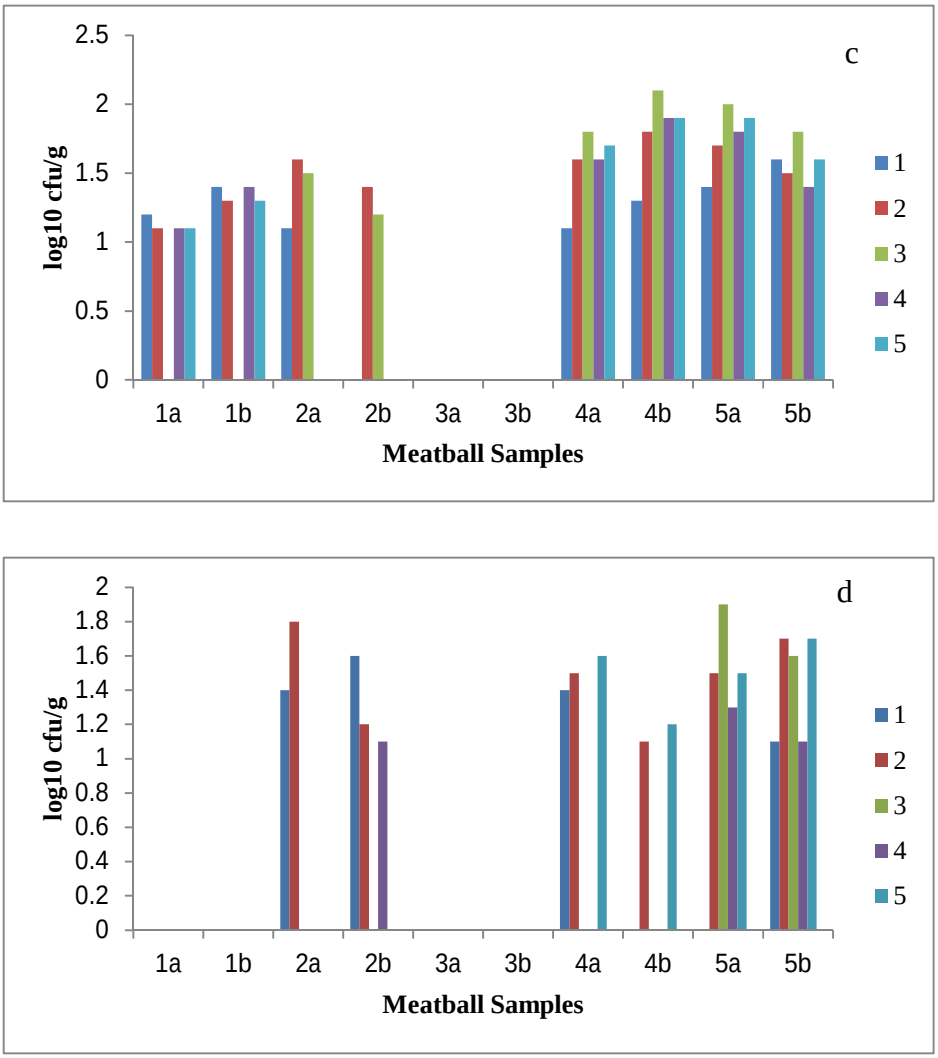


Figure 1. a: Number of total mesophilic aerobic bacteria, b: Number of Psychrophilic bacteria, c: Number of Enterobacteria, d: Number of coagulase positive *S. aureus*, 1a: First group meatballs taken from the first restaurant, 1b: Second group meatballs taken from the first restaurant, 2a: Second group meatballs taken from the first restaurant, 2b: Second group meatballs taken from the second restaurant, 3a: First group meatballs taken from the third restaurant, 3b: Second group meatballs taken from the third restaurant, 4a: First group meatballs taken from the fourth restaurant, 4b: Second group meatballs taken from the fourth restaurant, 5a: First group meatballs taken from the fifth restaurant, 5b: Second group meatballs taken from the fifth restaurant.

This study was performed to investigate the microbiological quality of Sivas kofte which is among conventional foods. There have been some researches regarding the microbiological qualities of raw and cooked meatballs and the researchers reported low qualities (*Sarımehmetoğlu et al., 1998, Soyutemiz, 1999, Yıldız et al., 2004; Kök et al., 2007*). During the handling, packaging, or serving of cooked products, some low level of contamination invariably occurs on the surface of the products from equipment and food handlers (*Johnston* and *Tompkin*, 1992).

It was determined that the number of total mesophilic aerobic bacteria determined in the meatball samples ranges between 2.7 and 4.9 $\log_{10}$ cfu/g. It was observed that this finding is lower than those reported by *Kıvanç* and *Kunduhoğlu* (1996) ($4.35x10^7$ cfu/g in Eskişehir) and *Yıldız et al.* (2004) ($5.6x10^5$ cfu/g in İstanbul) whereas it is closer to those found in a study performed by *Hampikyan et al.*, (2008) in İstanbul ($1.6x10^2$-$3.8x10^5$ cfu/g).

In a study performed in Ankara by *Aycicek et al.* (2005) in 17 (11.8 %) of 144 meatball samples coagulase positive *S. aureus* was determined at a level of 3.7-4.1 $\log_{10}$ cfu/g. Moreover, *Hampikyan et al.* (2008) reported coagulase positive *S. aureus* in 4 (20 %) of the 20 meatball samples between $<10^2$-$2.6x10^4$ cfu/g and *Gülmez et al.* (2005) determined that 4 (10 %) grilled meatball samples were contaminated by *S. aureus* over 10^2 cfu/g.

As it is well known, food handlers carrying enterotoxin-producing *S. aureus* in their noses or on their hands are regarded as the main source of food contamination, via manual contact or through respiratory secretions (*Argudin et al.* 2010). Some beef patties (14.8%) showed absence of *S. aureus*. The presence of small number of *S. aureus* is not uncommon (*Adams* and *Moss*, 2000). Human contact with cooked food invariably adds *S. aureus* at levels 10^1 or 10^2 to many sample units (*Surkiewicz*, 1973). Such levels are harmless but offer sufficient inoculum for growth (*Johnston* and *Tompkin*, 1992). The detection of *S. aureus* in beef patties, could have resulted result from food handlers, animal or environmental sources (*Lancette* and *Tatini*, 1992). In processed foods, in which *S. aureus* is destroyed by processing, its presence usually indicates contamination from the skin, mouth or nose of food handlers. An average prevalence of 19.8% *S. aureus* was found in 10 ready - to - eat consumer food (*Adesiyun et al.* 1995). Also, in this study, it was observed that Sivas kofte sample was in compliance with the limit values stated in the Microbiological Criteria of Turkish Food Codex (*RG, 2011*). Determining *S. aureus* in cooked products reveals both the inadequacy of thermal treatment applied onto the product and the necessity for staff hygiene. *S. aureus* contamination in cooked products is usually due to by employees' hands.

It was determined that the number of psychrophilic bacteria which determines the shelf life of the product changes between 1.6 and 3.8 $\log_{10}$ cfu/g. *Soyutemiz* (1999) determined the number of psychrophilic bacteria as $2.73x10^8$ cfu/g whereas *Kıvanç* and *Kunduhoğlu* (1996) found it as $3.88x10^5$ cfu/g for

cooked meatballs. It can be seen that the findings obtained in this study are lower than those reported earlier.

No *E. coli* was found in any of the Sivas kofte samples. *Hampikyan et al.*, (2008) determined coliform group microorganisms in 8 (40 %) of the 20 meatball samples ranging from 10^1 to 10^4 cfu/g. They found that 3 (15 %) samples contained *E.coli* at levels changing between 10^1-10^3 cfu/g. *Soyutemiz* (1999) determined an average number of coliform bacteria between 10^4 and 10^5 cfu/g. E coli was found in 33.3 % of these meatball samples. In their study, *Yıldız et al.,* (2004) found the number of coliform bacteria as 5.2 x10^3. *E.coli* was determined in 32 % (24/75) of the samples.

Davidson et al. (2000) reported coliform and *E. coli* at the level of 1.2x10^4 and 4.8x10^3, respectively. These results show that the microbial quality of ground meat vary depend on the technique used to slaughter animalsi contaminations may accur during evisceration of the internal organs, conditions of storage, personal hygiene.

According to Turkish Food Codex, *Salmonella* should not be detected in 25 g of the food samples. Improper preparation and handling of foods at food service establishments are primary factors in *Salmonella* outbreaks (*Jay*, 1992). In recent studies, authors reported data related to the contamination of minced meats with *Salmonella* and other foodborne pathogen bacteria in Turkey (*Soyutemiz,* 1999; *Gülmez et al.,* 2005, *Hampikyan et al.,* 2008) and other countries (*Parisi et al.*, 2010; *Wojcik et al.*,2010).No *Salmonella* spp. was determined in any of the Sivas kofte samples. This finding is in compliance with those reported by *Soyutemiz* (1999), *Gülmez et al.* (2005), *Hampikyan et al.* (2008). This result is considered as positive for public health.

Outbreaks of human salmonellosis, resulting from ingestion of animal originated foods contaminated with S. Typhimurium, have been reported in many countries (*Ethelberg et al*., (2008); *Mank et al*., (2010)).

Conclusion

In conclusion, though it differs according to the place where they were taken from, the microbiological quality of Sivas kofte in restaurants of Sivas were found suitable according to the Microbiological Criteria Regulation of Turkish Food Codex.

Acknowledgment

This study was a poster presentation at the IIIrd Symposium of the Traditional Foods, May, 10-12, 2012 in Konya, Turkey.

“Sivas kofte” i ispitivanje mikrobiološkog kvaliteta

O. P.Can, S. Şahin, M. Erşan, F. Harun

Rezime

Cilj ovog istraživanja je bio da se ispita tradicionalni proizvod od mesa iz pokrajine Sivas – “sivas kofte” u pogledu mikrobiološkog kvaliteta. Uzorci proizvoda koji se prodaje na tržištu su ispitani sa stanovišta mikrobiološkog kvaliteta (150 komada kuvanih uzoraka proizvoda – kofte, koji su uzeti iz 5 najpopularnijih restorana). Uzorci su analizirani u pogledu ukupnih mezofilnih aerobnih bakterija, enterobakterija, *E. coli*, koagulaza pozitivnih *S. aureus*, *Salmonella* spp. i psihofrilnih bakterija. Uzorci proizvoda spremnog za konzumiranje/serviranje su ispitani i dobijeni su sledeći rezultati: ukupan broj mezofilnih aerobnih bakterija, enterobakterija, koagulaza pozitivnih *S. aureus*, i psihofrilnih bakterija - 2.7-4.9 $\log_{10}$ cfu/g, <10 - 2.1 $\log_{10}$ cfu /g, <10 - 1.9 $\log_{10}$ cfu /g, 1.6- 3.8 $\log_{10}$ cfu /g, respektivno. .*E. coli* i *Salmonella* spp. nisu utvrđene ni u jednom uzorku. Kao rezultat ispitivanja, utvrđeno je da su „sivas kofte“ – spremne za konzumiranje, odgovaraju turskom standardu odnosno Pravilniku koji se odnosi na mikrobiološki kvalitet - Turkish Food Codex Cominiquate Microbiological Criteria, uprkos razlikama u mikrobiološkom kvalitetu na različitim lokacijama u pokrajini Sivas.

References

ADAMS M.R., MOSS M.O. (2000): Food Microbiology. Royal Society of the Chemistry, Cambridge, UK, 479.

ADESIYUN A.A. (1995): Bacteriologic quality of some Trinidadian ready - to - eat consume foods and drinks and public health risks to consumers. J. Food Protection, 58, 6, 651- 655.

ANAR Ş. (2010): Et ve Et Ürünleri Teknolojisi. Dora Basım Yayın Dağıtım, Bursa.

ARGUDIN M. A., MENDOZA M. C., RODICIO M. R. (2010): Food poisoning and *Staphylococcus aureus* enterotoxins. Toxins, 2, 1751-773.

AYCICEK H., CAKIROGLU S., STEVENSON T.H. (2005): Incidence of *Staphylococcus aureus* in ready to eat meals from military cafeterias in Ankara, Turkey. Food Control, 16, 531-534.

BAŞKAYA R., KARACA T., SEVINÇ I., ÇAKMAK O., YILDIZ A., YORUK M. (2004): İstanbul’da Satışa Sunulan Hazır Kıymaların Histolojik, Mikrobiyolojik ve Serolojik Kalitesi. YY Üniv Vet Fak Derg, 15, 1-2, 41-46.

DAVIDSON C., REILLY S.S., HARP E., GILLIAND S.S., MURIANA P.M. (2000): Incidence of Escherischia coli, Listeria monocytogenes, Campylobacter spp., and Salmonella spp. in ground beef and beef carcass surfaces in Oklahoma. Web page: www. Confex. Com/ift/99annual/abstracts/4679.htm.

EROL I. (2007): Gıda Hijyeni ve Mikrobiyolojisi, Pozitif Matbaacılık, Ankara.

ETHELBERG S., WINGSTRAND A., JENSEN T., SORENSEN G., MULLER L., LISBY M. (2008): Large outbreaks of Salmonella Typhimurium infection in Denmark in 2008. Eurosurveillance, 13, 3.

GÜLMEZ M., SEZER Ç., DUMAN B., VATANSEVER L., ORAL N., BAZ E. (2005): Lokantalarda tüketime sunulan bazı gıdaların ve içme sularının mikrobiyolojik kaliteleri. Kafkas Üniv Vet Fak Derg, 11, 1, 5-10.

HAMPIKYAN H., ULUSOY B., BINGOL E.B., ÇOLAK H., AKHAN M. (2008): İstanbul'da tüketime sunulan bazı ızgara tipi gıdalar ile salata ve mezelerin mikrobiyolojik kalitelerinin belirlenmesi. Türk Mikrobiyol Cem Derg, 38, 2, 87-94.

INTERNATİONAL COMMİSSİON ON MİCROBİOLOGİCAL SPESİFİCATİONS FOR FOOD (ICMFS) (1982): Microorganisms in foods. 1. Their signifiance and methods of enumeration. Univ Toronto Pres.

JAY J.M. (1992). Foodborne gastroenteritis caused by Salmonella and Shigella - Modern Food Microbiology, pp. 507-526.Chapman and hall, New York.

JOHNSTON R.W., TOMPKİN R.B. (1992): Meat and poultry products.In: Compendium of Methods for the Microbiological Examination of Foods, C. Vanderzant and D.F. Splittstoesser (Eds.), pp. 821-835. American Public Health Association, Washington, D.C.

KIVANÇ M., KUNDUHOĞLU B. (1996): Eskişehir'de tüketilen köftelerin mikrobiyolojik incelenmesi ve halk sağlığı açısından önemi. Anadolu Üniv Fen Fak Derg, 1, 5-15.

KÖK F., KESKIN D., BUYUKYORUK S. (2007): Çine köftelerinin mikrobiyolojik kalitelerinin incelenmesi. Erciyes Üniv Vet Fak Derg, 4, 1, 29-33.

LANCETTE G.A., TATINI S.R. (1992): Staphylococcus aureus. In: Compendium for the Microbiological Examination of Foods, C. Vanderzant and D.F. Splittstoesser (Eds.), pp. 533-592. American Public Health Association, Washington, D.C.

MANK L., MANDOUR M., RABATSKY-HER T., PHAN Q., KRASNITSKI J., BROCKMEYER J. (2010): Multiple-serotype Salmonella gastroenteritis outbreak after a receptionconnecticut, 2009. Morbidity and Mortality Weekly Report, 59, 1093-1097.

PARISI A., MICCOLUPO A., SANTAGADA G., PEDARRA C., DAMBROSIO, A. NORMANNO G. (2010): Detection of verocytotoxin-producing Escherichia coli (VTEC) in minced beef and raw milk by colony blot hybridization. Food Control, 21, 770-773.

PHILLIPS D., SUMNER J., ALEXANDER J., DUTTON K. (2001): Microbiological quality of Australian beef. J. Food Protect, 64,692-696.

RESMİ GAZETE (RG). (2010): 555 Sayılı Kanun Hükmünde Kararname gereği coğrafi işaretlerin korunmasına ilişkin tescil talebi ilanı-Sivas Köftesi. 27572, 5 Mayıs 2010, Başbakanlık Basımevi. Ankara.
RESMİ GAZETE (RG). (2011): Türk Gıda Kodeksi Mikrobiyolojik Kriterler Yönetmeliği, 28157, 29 Aralık 2011, Başbakanlık Basımevi, Ankara.
SARIMEHMETOĞLU B., KUPLULU Ö., KAYMAZ Ş. (1998): Hamburger ve İnegöl köftelerinden Escherichia coli O157:H7 izolasyonu. Ankara Üniv Vet Fak Derg, 45, 221-227.
SOYUTEMIZ G.E. (1999): Bursa'da satışa sunulan çeşitli hazır köftelerin hijyenik kalitesinin saptanması. Gıda, 24, 3, 163-169.
SURKIEWICZ B.F., HARRIS, M.E. , JOHNSTON R.W. (1973): Bacteriological survey of frozen meat and gravy produced at establishments under federal inspection. Applied Microbiology, 26, 574 -580.
TÜRK STANDARTLARI ENSTİTÜSÜ (TSE). (1992): TS 10581 Köfte-İnegöl Köfte-Pişmemiş, Ankara.
WOJCIK-STOPCZYRISKA B., JAKUBOWSKA B., SZOT K. (2010): Evaluation of microbiological quality of seasoning purchased in the retail network. Roczniki Panstwowego Zakladu Higieny, 61, 45-50.
YILDIZ A., KARACA T., ÇAKMAK O., YORUK M., BAŞKAYA R. (2004): İstanbul'da tüketime sunulan köftelerin histolojik, mikrobiyolojik ve serolojik kalitesi. YY Üniv Vet Fak Derg, 15, 1-2, 53-57.

Received 22 October 2012; accepted for publication 21 January 2013

Biotechnology in Animal Husbandry 29 (1), p 145-159 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 637.045:043:04'637.354
DOI: 10.2298/BAH1301145B

CORRELATION BETWEEN PROTEIN TO FAT RATIO OF MILK AND CHEMICAL PARAMETERS AND THE YIELD OF SEMI-HARD CHEESE

M. Bojanić Rašović[1], N. Nikolić[1], A. Martinović[1], V. Katić[2], R. Rašović,[3] M. Walcer[4], K. Domig[4]

[1] Biotechnical faculty, Podgorica, Montenegro
[2] Faculty of Veterinary Medicine, Belgrade, Serbia
[3] ZZ "Cijevna, Podgorica, Montenegro
[4] University of Natural Resources and Life Sciences, Vienna
Corresponding author: bojanic.m@t-com.me
Original scientific paper

Abstract: In order to obtain good cheese quality, the milk has to possess good physical-chemical properties and should originate from healthy cows. Since milk fat and milk protein are the main constituents of cheese, their proportion in milk is of particular importance for the product yield and quality. This paper describes studies on the protein to fat ratio of milk and the consequent influence on the chemical composition and yield of semi-hard cheese, commercially called "Montenegrin naturally dried cheese". The tests were conducted on six bulk milk samples and six cheese samples. The milk parameters were analysed by the Milkoscan 400 unit whereas chemical analysis of cheese and whey were carried out with the Milkoscan FT 120 device. The average composition of the cheeses was: 29.27% fat, 21.90% protein, 55.27% total dry matter and 0.78% sodium chloride. The mean value for the content of dry matter without fat was 26%, whereas the fat content of the cheese dry matter was 53.18%, the moisture content in cheese 44.73% and moisture content in fat-free matter 63.24%. There was a medium positive correlation between the ratio of fat to protein in milk and fat content in cheese (r = 0.60309), the ratio of fat to protein in milk and dry matter of cheese (r = 0.57103), weak positive correlation between the ratio of fat to protein in milk and cheese protein (r = 0.48067) and medium negative correlation between the ratio of fat to protein in milk and moisture content in cheese (r = -0.57103). Medium negative correlation was found between the ratio of protein to fat in milk and content of cheese fat (r = -0.56416), the ratio of protein to fat in milk and cheese protein content (r = -0.51899), the ratio of protein to fat in milk and dry matter of cheese (r = -0.53118) and medium positive correlation between the ratio of protein to fat in milk and moisture content in cheese (r = -0.53118). Ratio fat to protein in milk and the actual yield of cheese was determined as medium positive (r = 0.66459) and the ratio protein to fat in milk and the actual yield of cheese as

medium negative correlation (r = -0.67807). The protein to fat ratio in milk influences the decline of fat, protein, dry matter and yield of cheese and increase moisture content in cheese.

Key words: fat, protein, ratio, cheese, yield

Introduction

The chemical composition of milk is one of the major factors concerning the production of fermented dairy products. Since milk fat and protein are main constituents of cheese, the product quality is heavily influenced by their concentrations in milk. Chemical composition and physical properties of milk can vary in a certain range and influenced by various factors and reflected in the properties of cheese. In first place, a good quality and unchanging products can be achieved by using immaculate and standardised milk. The main component determining the quality of milk used for cheese production is the protein content. Milk high in protein, especially casein, results in a high yield and good quality cheese products. The higher the ratio of casein to whey protein in milk, the more suitable it is for cheese production. The fat content is important as well, but especially the comparison with the protein or casein content is of significance. The common proportion of casein to fat is ranging from 0.64 to 0.72 *(Amenu and Deeth,* 2007). The quality of cheese, its caloric and nutritional value, physical properties and the chemical composition of curd and cheese dough depends to a large extent on the fat content. If milk contains more fat than needed for a particular cheese, the whey draining off the curd contains higher fat concentrations, which constitutes a significant economic loss *(Pejić, 1956.).* Variations in quantity and composition of milk are either caused by dominant inheritance (55%) or by paragenetic factors, among which the most important is feed. Other considerable paragenetic factors are breed, type of feed, stage of lactation, age of animal, milking quality, health, environmental conditions (temperature, humidity, air flow), way of keeping, etc. An indicator of disturbance in the composition of milk is the ratio protein to fat, which should range from 0.8 to 0.9 *(Dozet et al., 1996; Adamović et al., 2003).* Heritability for the protein to fat ratio is 0.79, indicating the possibility of its genetic changes *(Vos and Groen, 1998).* The fat content in milk can vary within wide limits, from 2.5% to 5% or sometimes even more. The content of protein in the milk varies to lesser extent in comparison to the milk fat content. The biggest fraction within the protein content is covered by casein, with about 3% overall, whereas albumin and globulin are less frequent, with together about 0.5% *(Mišić-Čubrić, 1971).* The components of milk are very sensitive indicators for changes or problems in nutrition and the health of cows. The fat content in milk provides information about the creation of acetic acid in the rumen.

Changes in the ratio fat to protein, with protein content equal to or greater than the fat content, indicate that the rumen is not performing properly. The risk of ketosis in cows is determined by the ratio of protein to fat less than 0.75 *(Silva-del-Río et al., 2011).* Reduced fat content can be a problem for entire herds as well as only for individual cows. A ratio of fat to protein in milk from cows of Holstein breed less than 1.0 indicates problems of reduced fat content or syndromes of low fat content in dairy herds. In cases of severe reduction of milk fat content, the ratio fat to protein in milk was lower than 0.8. Inadequate content of cellulose fibre in the diet is the most common reason of low fat content in milk obtained from entire dairy herds. Depending on the diet, the milk fat content can deviate up to 1.0% from the usual value, while the protein content is rarely changing by more than 0.1 to 0.4%. Production of milk with increased fat content is economically not justified hence the total milk production is reduced. Reduced protein content in milk (less than 3.0% for Holstein cows) is usually caused by insufficient amounts of carbohydrates in the diet and less due to lack of protein in the feed. Changes in the concentrations of protein and fat in milk significantly influence the composition of the cheese and its yield. The increase of ratio protein to fat in milk significantly raises the protein, calcium and phosphorus contents in cheese and has an extensive impact on reducing the moisture content of fat-free matter, fat in dry matter and salt content. Those criteria reveal the importance of standardizing protein and fat contents in order to avoid poor quality and incompatibility with product specifications. By applying different ratios of protein to fat in milk, combined with other changes concerning the production, new types of cheeses can be obtained *(Guinee et al., 2007).* Minding the significance of ratio protein to fat in milk intended for cheese production, the objective is to examine the correlation between the ratio of protein to fat in milk on the chemical composition and the yield of extra hard cheese.

Material and methods

Tests were conducted on 6 samples of bulk milk and 6 samples of cheese examined after pressing. Chemical analysis of milk was carried out on the device Milcoscan 400 and for cheese and whey Milcoscan FT 120 device was used.

Actual cheese yield was calculated as the weight of cheese obtained from 100L of milk, expressed in percentages.

For the purpose of comparing the yield of cheeses with different moisture contents, yield was calculated on the straight-out moisture of 38.5% by the following formula: $Y_{ma} = Y_a \times 100 \div M_a/100 \div M_r$, where M_a is the actual moisture content in cheese and M_r reference value for moisture content (38.5%). In order to compare the obtained yield in relation to the reference protein and fat content of milk, calculated actual yield of cheese was normalized to reference fat content (3.4%) and protein (3.3%), the following formula: $Y_{afpam} = Y_a \times F_{rm} + P_{rm}/F_{cm} + P_{cm}$,

where F_{cm} and P_{cm} values obtained for fat and protein content of milk, and F_{rm} and P_{rm} percentages of fat and protein in the reference milk for cheese production (3.4% and 3.3%) *(Guinee et al., 2006).*

Several statistical parameters were determined: mean (X), maximum (max) and minimum (min) value, standard deviation (SD) and correlation.

Determining the strength of correlation was done using Čebis's table *(Trbojević, 1986).*

Results and Discussion

Results of the chemical composition of cow bulk milk are shown in Table 1.

Table 1: Composition of bulk cow milk analysed by Milkoscan technique

Number of cheese samples	Fat (%)	Protein (%)	Lactose (%)	Dry matter without fat (%)	Ratio fat to protein of milk	Ratio protein to fat of milk	The content of protein and fat (g/kg milk)
6	3.91	3.27	4.34	8.34	1.19	0.84	71.80
7	3.77	3.24	4.29	8.27	1.16	0.86	70.10
8	3.82	3.28	4.31	8.32	1.16	0.86	71.00
9	3.90	3.28	4.30	8.31	1.18	0.84	71.80
10	3.80	3.20	4.25	8.18	1.18	0.84	70.00
11	3.72	3.26	4.27	8.26	1.14	0.88	69.80
$\overline{X}$	3.82	3.25	4.29	8.28	1.16	0.85	70.75
max	3.91	3.28	4.34	8.34	1.19	0.88	71.80
min	3.72	3.20	4.25	8.18	1.14	0.84	69.80
SD	0.074	0.030	0.031	0.057	0.018	0.016	0.912

Table 1 shows the results of the chemical analysis of the milk samples. The mean value for the fat content was 3.82%, 3.25% for the protein content, 4.29% for the lactose content and 8.28% for the dry matter content without fat. The mean value for the ratio protein to fat amounted to 0.85 and the ratio fat to protein of milk 1.16. The mean total fat and protein content amounted to 70.75 g/kg of milk.

Table 2: Chemical parameters and yield of cheese, dry matter of whey

Number of cheese samples	Fat (%)	Proteins (%)	Total dry matter (%)	NaCl (%)	Actual cheese yield (%)	Yield moisture-adjusted (to 38.5%)	Yield of cheese normalized to reference levels of protein (3.4%) and fat (3.3%) (%)	Dry matter content in whey (%)
6	30.62	21.82	56.81	0.81	11.51	10.99	10.70	7.11
7	29.18	21.99	55.41	0.88	10.95	10.46	10.47	6.98
8	29.50	21.69	54.65	0.75	11.40	10.89	10.71	6.99
9	28.03	22.02	53.84	0.75	11.75	11.22	10.93	7.03
10	30.26	22.11	56.66	0.71	11.07	10.57	10.62	6.85
11	28.03	21.75	54.27	0.76	10.88	10.39	10.44	6.97
$\overline{X}$	29.27	21.90	55.27	0.78	11.26	10.75	10.64	6.99
max	30.62	22.11	56.81	0.88	11.75	11.22	10.93	7.11
min	28.03	21.69	53.84	0.71	10.88	10.39	10.44	6.85
SD	1.090	0.167	1.245	0.060	0.346	0.330	0.180	0.085

The results in Table 2 reveal that the maximum value for the fat content in cheese after pressing was 30.62%, the minimum 28.03% and the mean value 29.27%. The maximum value for protein content in cheese amounted to 22.11%, the minimum to 21.69% and in average to 21.90%. The maximum value for the content of total dry matter in cheese was 56.81%, the minimum 53.84% and the mean value 55.27%. The maximum value for the salt content was 0.88%, the minimum 0.71% and the mean value 0.78%. The mean value of the actual yield of cheese amounted to 11.26% and the yield adjusted to 38.5% moisture was 10.75%. The mean value for dry matter content in whey was 6.99%.

Table 3: Results of examination content of dry matter without fat, fat in dry matter, cheese moisture and moisture in fat-free dry matter

Number of cheese samples	Dry matter without fat (%)	Fat in dry matter of cheese (%)	Moisture in the cheese (%)	Moisture in fat free dry matter (%)
6	26.19	53.90	43.19	46.10
7	26.23	52.67	44.59	47.34
8	25.15	53.98	45.35	46.02
9	25.81	52.06	46.16	47.94
10	26.40	53.41	43.34	46.60
11	26.24	53.09	45.73	48.35
$\overline{X}$	26.00	53.18	44.73	63.24
max	26.4	53.98	46.16	64.33
min	25.15	52.06	43.19	62.14
SD	0.462	0.740	1.245	0.852

The results in Table 3 show that the mean value for the content of dry matter without fat is 26.00%, the fat content of cheese dry matter 53.18%, the moisture content in cheese 44.73% and the moisture content in fat free dry matter 63.24%.

Table 4: Classification of cheese samples on fat content in dry matter and moisture content in fat free matter

Number of samples	Fat in dry matter of cheese (%)	Moisture content in fat free matter (%)	Classification of cheese regarding % fat in dry matter	Classification of cheese to rheological characteristics (the percentage of moisture content in fat free dry matter of cheese)
6	53.90	62.25	full-fat	Semi-hard
7	52.67	62.96	full-fat	Semi-hard
8	53.98	64.33	full-fat	Semi-hard
9	52.06	64.14	full-fat	Semi-hard
10	53.41	62.14	full-fat	Semi-hard
11	53.09	63.54	full-fat	Semi-hard
$\overline{X}$	53.18	63.24		
max	53.98	64.33		
min	52.06	62.14		
SD	0.400	0.852		

The results in Table 4 show that all investigated samples of cheese belong to the full-fat cheeses by the amount of fat in dry matter, as they contain more than 45% milk fat and the rheological characteristics are typical for semi- hard cheeses since they contain 54% to 69% of water in fat-free dry matter of cheese. Regulations on quality and other requirements for milk, dairy products, composite dairy products and starter cultures can be found in Gazette SRJ No 26/2002 and Gazette SCG, No 56/2003.

The results in Table 5 show that there is a medium positive correlation between the ratio fat to protein in milk and fat content in cheese, the ratio fat to protein in milk and dry matter in cheese, ratio fat to protein in milk and actual yield cheese and a medium negative correlation between the ratio fat to protein and moisture content in cheese. Medium negative correlation was found between ratio protein to fat and the fat content in cheese, the ratio protein to fat and protein content in cheese, the ratio protein to fat and dry matter of cheese, the ratio protein to fat and the actual yield of cheese and medium positive correlation of ratio protein to fat and moisture content in cheese.

Table 5: Results of correlation between ratio protein to fat in milk and chemical parameters and yield of cheese

Correlation between:	Strength of correlation	Correlation coefficient
Cheese fat content and moisture content in cheese	Very strong negative	-0.93040
Fat content in dry matter of cheese and moisture content in cheese	Medium negative	-0.55447
Fat content in dry matter of cheese and moisture content in the fat-free cheese matter	Strong negative	-0.79894
Fat content in dry matter of cheese and moisture content in the cheese	Medium positive	0.59500
Ratio fat to protein of milk and fat in cheese	Medium positive	0.60309
Ratio fat to protein of milk and fat in dry matter of cheese	Weak positive	0.07886
Ratio fat to protein of milk and cheese dry matter	Medium positive	0.57103
Ratio fat to protein of milk and protein content in cheese	Weak positive	0.48067
Ratio fat to protein of and moisture content in cheese	Medium negative	-0.57103
Ratio fat to protein of milk and moisture content in the fat-free cheese matter	Medium negative	-0.51673
Ratio fat to protein of milk and yield adjusted to 38.5% moisture	Medium positive	0.66193
Ratio fat to protein and actual yield of cheese	Medium positive	0.66459
Ratio fat to protein and yield of cheese normalized to reference levels of protein and fat	Medium positive	0.66360
Protein content of cheese and moisture content of cheese	Weak negative	-0.27880
Ratio protein to fat of milk and fat content in cheese	Medium negative	-0.56416
Ratio protein to fat of milk and fat content in dry matter of cheese	Weak negative	-0.02915
Ratio protein to fat of milk and protein content in cheese	Medium negative	-0.51899
Ratio protein to fat of milk and dry matter of cheese	Medium negative	-0.53118
The ratio protein to fat of milk and content of fat-free dry matter of cheese	Weak negative	-0.10092
Ratio protein to fat of milk and moisture content in cheese	Medium positive	0.53118
Ratio protein to fat of milk and moisture content in dry matter without fat cheese	Weak positive	0.48679
Ratio protein to fat of milk and moisture content in the fat-free cheese matter	Weak negative	-0.23407
Ratio protein to fat of milk and the actual yield of cheese	Medium negative	-0.67807
Ratio protein to fat of milk and yield adjusted to 38.5% moisture	Medium negative	-0.67548
Ratio protein to fat of milk compared to yield of cheese normalized to reference levels of protein and fat	Medium negative	-0.69300
Total fat and milk protein (g/kg) and the actual yield of cheese	Very strong positive	0.96174
Ratio protein to fat of milk and fat in dry matter of cheese	Weak positive	0.09399

Table 6: **Results of correlation between chemical parameters of cows bulk milk, cheese and whey**

Correlation between	Intensity of correlation	Values for coefficient of correlation
Fat content in milk and whey dry matter	Medium positive	0.61380
Protein content in milk and whey dry matter	Strong positive	0.80584
Dry matter content of milk and whey dry matter	Very strong positive	0.93568
Protein content of cheese and whey dry matter	Weak negative	-0.45286
Fat content of cheese and whey dry matter	Weak negative	-0.00108
Dry matter content of cheese and whey dry matter	Weak negative	-0.09145
Ratio protein to fat of milk and whey dry matter	Weak negative	-0.23407
Ratio fat to protein of milk and whey dry matter	Weak positive	0.26731
The fat content of milk and whey fat content	Medium positive	0.60570
The fat content of milk and whey protein content	Weak negative	-0.18355
Protein content of milk and whey protein content	Medium positive	0.55103
Protein content of milk and whey fat content	Weak negative	-0.19837

The results in Table 6 show that there is a very strong positive correlation between the dry matter content of milk and the whey dry matter and medium positive correlation between milk fat content and dry matter content of the whey.

During cheese production the milk components get concentrated. Especially the fat and protein contents determine the yield of cheese production, thus affecting the efficiency and effectiveness profoundly. Cheese yield is determined by many factors, such as the composition of milk, milk pre-treatment, the type of rennet, the curd processing and so on. Some properties of milk, particular solubilisation of proteins by the proteolytic activity of plasmin, somatic cell count, pH value, mineral content and urea content affect the yield of cheese production as well *(Verdier-Metz et al., 2001).*

Fat content in dry matter and moisture content in the fat-free matter are the main determinants for quality of Cheddar cheese and are determined by levels of moisture and protein in cheese. Variations in ratio protein to fat in milk influence the cost-effectiveness of milk production, the composition and quality of cheese. Vast seasonal deviations of ratio protein to fat (~ 0.72 to 1.0) in milk give rise to differences in moisture content in the fat-free matter of cheese, as well as differences in consistency and texture of cheese. The ratio protein to fat in milk was negatively correlated with moisture content in the fat-free matter of cheese and cheese firmness *(Guinee et al., 2007).* During the study of this work a negative correlation between the ratio milk protein to fat and the moisture in fat-free substance of cheese was obtained (-0.23407) (Table 5). The opposite effect of ratio protein to fat in milk and moisture in the fat-free matter of cheese may due to fat

droplets supressing effect on the permeability of the milk and the curd syneresis. By examining correlations between the ratio protein to fat and moisture in the cheese, we got a medium positive correlation (0.53118). These results are consistent with the results of *Guinee et al., (2007)*, who also received a positive correlation of ratio protein to fat in milk and cheese moisture. These results authors explained thereby that on the moisture content in cheese, besides of fat droplets, influence other factors, such as various technological processes in cheese production, e.g. increasing the pasteurization temperature, pre-acidification of milk before adding rennet, the use of selected starter cultures that produce exopolysaccharides, changes in temperature, pH of the curd, the release of whey, processing curd, etc. *Fenelon and Guinee (1999)* reported that increasing protein content in milk reduces the moisture content in cheese. Despite the higher actual yield and reduced water retention with reduced ratio proteins to fat of milk, the yield normalized to relative humidity decreases. In the tests performed in this study the results are similar. The actual yield increases, but the normalized yield decreases (Table 2).

The ratio protein to fat in milk significantly affects cheese yield and the percentage of fat and water retention in cheese. In particular, this matter influences the fat content in dry matter of cheese and the water content of fat-free matter. This indicates the importance of standardising the ratio protein to fat in order to avoid poor quality and non-compliance with the product specifications. By applying different ratios of protein to fat in milk, combined with other changes in production, new types of cheeses can be obtained.

A ratio of protein to fat in milk between 0.70 and 0.85 results in significantly less fat retention during cheese production compared to cheese produced from milk with a ratio protein to fat between 0.88 and 1 or between 1.01 and 1.15. The increase of ratio protein to fat in milk leads to a significant reduction in the actual yield of cheese *(Guinee et al. 2007).* These results are consistent with the medium negative correlation between the ratio protein to fat and actual cheese yield gained during this study (-0.67807), (Table 5), which can be explained by the higher content of fat and moisture in the cheese.

The ratio of milk fat to casein has a huge effect on the physical and chemical properties of cheese as well as on the fat content in cheese itself, since it is responsible for the amount of milk fat that remains in the curd. Under the same production conditions and the same technological procedure, cheeses from milk with higher fat content have a finer, softer consistency, while the cheeses obtained from milk with lower fat content have a tough consistency. The amount of moisture in the fresh cheese is of great information value regarding its consistency and process maturity. Cheeses with higher moisture content have distinct milk sugar fermentation. The desired percentage of moisture in the cheese curd can be achieved by processing of milk and curd, as well as by molding, pressing and salting of cheese.

In curd obtained from milk with a higher content of fat, moisture and whey slower segregates. Milk fat mechanically affects the detraction of moisture. Thus the adjustment of milk fat is not only important from an economic point of view but also from a technological point of standardization of properties and quality of the products. Utilization of milk proteins in cheese is in line with the attainment or transfer of dry matter in cheese, in particular fat-free dry matter *(Pejić, 1950).*

Agabriel et al. (1991.) figured out that the ratio of fat to protein in milk from dairy herds from 62 farms ranged between 1.10 and 1.25. This ratio was deviating depending on the farm and month of the investigation. Small variations in the ratio in the months of testing on the same farm can be explained by diverse nutrition. In this study the mean value for the ratio fat to protein in milk was 1.16 and the mean value for the ratio protein to fat in milk was 0.890. *Čejna and Chládek (2005)* examined of the milk of Holstein cows on day 25, 45, 73, 101, 133, 166, 199, 224, 253 and 280 of lactation and the corresponding values for ratio of fat to protein were determined as 1.91, 1.45, 1.38, 1.28, 1.22, 1.14, 1.26, 1.21, 1.09, and 1.18. The quality of the curd obtained from milk in the first stage of lactation was lower. The high value for the ratio fat to protein in the first phase of lactation is caused by deficiency of energy.

The ratio protein to fat of milk for cheese production in Ireland during the period from 2001 to 2003 year, varied from 0.84 to 1.02 while the protein content varied from 2.99% to 3.59% and fat content from 3.26% to 4.2%, depending on the farm, the season and the year. Research shows that for a number of cheeses, produced in Ireland or the UK, ratio protein to fat varies from 0.68 to 0.85, which indicates that the ratio protein to fat in milk varies from 0.81 to 1.02 and supposes that milk for Cheddar cheese production is not generally standardised by ratio protein to fat. Studies in Scotland show similar variations in ratio protein to fat of cheese with values from 0.79 to 0.97. On the contrary investigations concerning milk from the United States show a much lower variation of ratio protein to fat. Variations in ratio protein to fat in milk are the result of natural and seasonal variations of fat protein, casein and lactose in milk, as well as results of stage of lactation, nutrition, etc. *(Guinee et al., 2007).*

Different fat content, and therefore a different ratio protein to fat in milk used in the production of low fat and full fat cheeses significantly affects the composition, yield, rheological and sensory characteristics of cheese *(Guinee et al., 2007).*

Phelan (1981) considers that there is no correlation between the ratio protein to fat in milk (0.9 to 1.01) and fat in dry matter of Cheddar cheese and that variations in fat loss via whey have a greater impact on fat in dry matter of cheese then ratio protein to fat in milk. In the tests performed in this study no correlation between the ratio protein to fat and fat in dry matter of cheese was evident (Table 5). Retention of fat in cheese is influenced by several other unidentified components or properties of milk.

The content of calcium and phosphorus in cheese produced from milk with a ratio protein to fat of 0.70 to 0.85 are significantly lower than in cheeses manufactured from milk with ratio protein to fat of 1.01 to 1.15. This decrease can be explained by reduced protein content regarding the reduced ratio protein to fat in milk.

The four parameters playing a key role in determining the quality of cheese are salt content, moisture content in the fat-free matter, pH value and fat content in dry matter of cheese, which are influencing each other. Hence it is necessary to do some further investigation of the factors influencing the relationship between these four parameters and thus the quality of cheese. Due to the higher fat content in milk, the mean actual yield of cheese obtained from milk with ratio protein to fat of 0.70 to 0.85 was significantly higher (1.0 to 1.4 kg/100kg milk) compared to the cheese obtained from milk with ratio protein to fat of 0.88 to 1.00 or 1.01 to 1.15. Changes in ratio protein to fat in milk may have a different impact on the production of cheese. Natural seasonal variations of ratio protein to fat in milk range from 0.8 to 1.0. This range offers good opportunities for the optimization of cheese production *(Guinee et al. 2007.).*

Examining the effect of different concentrations of protein and fat milk in the range of 3-4%, *Lou and Ng-Kwai-Hang, 1992b*, found that higher yield of cheese is obtained from milk with higher fat and protein content in milk. A bigger quantity of cheese is obtained at higher ratio protein to fat or casein to fat. The increase in milk fat content leads to an increase in cheese moisture content by 1.23% to 1.37%, depending on the protein content in milk

These results are consistent with the results from this study, where a medium positive correlation could be observed between the ratio fat to protein in milk and fat content in cheese (0.60309), the ratio of fat to protein in milk and dry matter of cheese (0.57103), the ratio fat to protein in milk and moisture content in cheese (0.51678) and a weak negative correlation between the ratio fat to protein in milk and cheese protein (0.48067). Medium negative correlation was found between the ratio protein to fat in milk and fat cheese (-0.56416), the ratio protein to fat in milk and protein cheese (-0.51899), the ratio protein to fat in milk and moisture content in cheese (-0.56140) and medium negative correlation between the ratio protein to fat in milk and dry matter of cheese (-0.53118). The correlation between the ratio fat to protein in milk and the actual yield of cheese was medium positive (0.66459) (Table 5).

Cheese obtained from milk with higher protein concentration (3.0%, 3.2%, 3.4%, 3.6%, 3.8%, 4.0%) contains more protein, less fat and less total dry matter. Cheese obtained from milk with higher fat content has increased fat and decreased protein content. The higher content of fat in milk leads to lower retention of fat in cheese and thus extensive losses via whey *(Lou and Ng-Kwai-Hang, 1992a).* These results are consistent with the results from this study where medium positive correlation between fat content in milk and whey dry matter and a strong positive correlation between fat content of milk and whey dry matter were obtained (Table

6). Higher protein content in milk leads to greater retention of fat in cheese, or a small loss in the whey. For every percentage increase in milk fat content, the fat content in cheese increases by 4.22% and the protein content in cheese decreases by 2.61%. For every percentage increase in milk protein content, the protein content of cheese increases by 2.35% and the fat content reduce by 6.14%. Cheese produced from milk with a ratio protein to fat near 0.9 has at least 50% fat in dry matter of cheese *(Lou and Ng-Kwai-Hang, 1992a).* The investigations in this study confirm this observation (Table 4).

The ratio fat to protein in milk could be an indicator of the ability of cows to adapt to the demands of milk production and reproductive efficiency in the post partum period resulting in a prolonged postpartum period. *(Podpečan et al., 2010).* The ratio of fat to protein of 1.1 provides over 90% reliable results for the identification of cows in which the interval from calving to conception is shorter than 120 days. In cases where the ratio fat to protein is bigger than 1.44, the reliability of the identification of the above mentioned cows is more than 90%. Ratio fat to protein in cow's milk can also be used in the detection of subclinical ketosis. Subclinical ketosis is indicated when the ratio fat to protein in milk exceeds a value of 1.5 for cows milking 33 to 50 kg *(Gantner et al., 2008).* Dairy cows with a ratio of milk protein to fat less than 0.75 are at risk of ketosis *(Silva-del-Río et al., 2011).* Increase of milk fat content and increase of ratio fat to protein may be important in assessing the risk of dislocation abomasum *(Geishauser et al., 1998).*

Conclusion

A medium positive correlation was evident between the ratio fat to protein in milk and fat content in cheese (0.60309), the ratio fat to protein in milk and dry matter of cheese (0.57103), as well as a weak positive correlation between the ratio fat to protein in milk and protein of cheese (0.48067) and medium negative correlation between the ratio fat to protein in milk and moisture content in cheese (-0.57103). Medium negative correlation was apparent between the ratio protein to fat in milk and cheese fat (-0.56416), the ratio protein to fat and protein cheese (-0.51899), ratio protein to fat in milk and dry matter of cheese (-0.53118) and a medium positive correlation between the ratio protein to fat in milk and moisture content in cheese (0.53118). The correlation between the ratio fat to protein in milk and the actual yield of cheese was medium positive (0.66459) and the correlation between ratio protein to fat and the actual yield of cheese medium negative (-0.67807). The ratio protein to fat in milk has a negative impact on the content of fat, protein, dry matter, the actual yield of cheese, the moisture content in dry matter without fat, and a positive impact on the moisture content of cheese. According to the regulations on quality and other requirements for milk, dairy products, composite dairy products and starter cultures in Gazette SRJ No. 26/2002

and Gazette SCG No 56/2003 cheeses were classified as full fat, semi-hard cheeses.

Korelacija između odnosa proteina i masti u mleku i hemijskih parametara i randmana polutvrdog sira

M. Bojanić Rašović, N. Nikolić, A. Martinović, V. Katić, R. Rašović, M. Walcer, K. Domig

Rezime

Da bi se dobio sir dobrog kvaliteta mleko mora da ima dobre fizičko-hemijske osobine i da potiče od zdravih krava. S obzirom da su mlečna mast i proteini osnovni sastojci sira, to je njihov udeo u mleku od izuzetnog značaja za kvalitet proizvoda. U radu je ispitivan uticaj odnosa proteina i masti zbirnog mleka krava na hemijski sastav i randman polutvrdog sira, komercijalnog naziva „Crnogorski prirodno sušeni sir“, proizvoda sirare „ZZ“Cijevna“ u Podgorici. Ispitivanja su sprovedena na 6 uzoraka zbirnog mleka krava i 6 uzoraka sira uzetog nakon presovanja. Hemijska ispitivanja mleka su rađena na aparatu Milcoscan 400, a hemijska ispitivanja sira i surutke na aparatu Milcoscan FT 120. Srednja vrednost sadržaja masti u siru iznosila je 29.23%, sadržaja proteina 21.85%, sadržaja ukupne suve materije 55.06% i sadržaja soli 0.80%. Srednja vrednost za sadržaj suve materije bez masti je iznosila 26,00%, za sadržaj masti u suvoj materiji sira 53.18%, za sadržaj vlage u siru 44.73 % i sadržaj vlage u suvoj materiji bez masti 63.24 %. Utvrđena je srednja pozitivna korelacija između odnosa masti i proteina mleka i sadržaja masti u siru (0.603091), odnosa masti i proteina mleka i suve materije sira (0.571035), niska pozitivna korelacija između odnosa masti i proteina mleka i proteina sira (0.48067) i srednja negativna korelacija između odnosa masti i proteina i sadržaja vlage u siru (-0.57103). Srednja negativna korelacija je utvrđena između odnosa proteina i masti u mleku i masti sira (-0.56416), između odnosa proteina i masti i proteina sira (-0.51899), između odnosa proteina i masti i suve materije sira (-0.53118) i srednja pozitivna korelacija između odnosa proteina i masti i sadržaja vlage u siru (-0.531184). Između odnosa masti i proteina mleka i stvarnog randmana sira utvrđena je srednja pozitivna (0.664594), a između odnosa proteina i masti i stvarnog randmana sira srednja negativna korelacija(-0.67807).
Odnos proteina i masti u mleku ima uticaja na smanjenje sadržaja masti, proteina, suve materije i randmana sira i na povećanje sadržaja vlage u siru.

References

ADAMOVIĆ M., LEMIĆ J., MILIĆ B., ADAMOVIĆ O., RADIVOJEVIĆ M. (2003): Novi rezultati o mogućnostima očuvanja sadržaja važnijih sastojaka mleka, Prehrambena industrija, 1-2, 143-149

AGABRIEL C., COUON J.B., MARTY G. (1991): Facteurs de variations du rapport des teneurs en matières grasses et protéiques du lait de vache: étude dans les exploitations des Alpes du Nord, Inra Prod. Anim., 4(2), 141-149

AMENU B., DEETH H.C. (2007): The impact of milk composition on cheddar cheese manufacture, Australian Journal of Dairy technology, 62 (3), 171-184

ČEJNA V., CHLÁDEK G. (2005):The importance of monitoring changes in milk fat to milk protein ratio in Holstein cows during lactation, Journal Central Europe Agriculture, 6 (4), 539-546

FENELON M.A., GUINEE T.P.(1999): The effect of milk Fat on cheddar cheese yield and its prediction, using modifications of the van slyke cheese yield formula, J Dairy sci, 82, 2287–2299

GANTNER V., POTOČNIK K., JOVANOVAC S., RAGUŽ N. (2008): Utjecaj supkliničke ketoze na dnevnu količinu i sastav mlijeka slovenskih Holstein krava, Krmiva 50, (5), 253-259

GEISHAUSER T. D., LESLIE K. E., DUFFIELD T. F.B., EDGE L V.(1998): An evaluation of protein/fat ratio in first DHI test milk for Prediction of subsequent displaced abomasum in dairy cows, can J vet res; 62, 144-147

GUINEE, T. P., B. T. O'KENNEDY, KELLY P. M.(2006): Effect of milk protein standardization using different methods on the composition and yields of Cheddar cheese. J. Dairy Sci. 89, 468–482

GUINEE C. T. P., MULHOLLAND E. O., KELLY J., AND CALLAGHAN D. J. O. (2007): Effect of protein-to-fat ratio of milk on the composition, manufacturing efficiency, and yield of cheddar cheese, J. dairy sci. 90, 110–123

LOU Y., NG-KWAI-HANG K.F.(1992a): Effects of protein and fat levels in milk on cheese and whey compositions, Food Research International, Volume 25 (6), 445-451

LOU Y.,NG-KWAI-HANG K.F. (1992b): Effects of protein and fat levels in milk on Cheddar cheese yield, Food Research International, Volume 25, Issue 6, 437-444.

MIŠIĆ- ČUBRIĆ- D. (1971): Suva materija mleka-važno merilo kvaliteta, Mljekarstvo, 21 (9), 194-200

DOZET N., ADŽIĆ N., STANIŠIĆ M., ŽIVIĆ N. (1996): Autohtoni mlječni proizvodi, Poljoprivredni institut, Podgorica

PEJIĆ O.(1956): Mlekarstvo, II deo, Beograd

PHELAN, J. A. (1981): Standardization of milk for cheesemaking at Factory level, J. Soc. Dairy Technol. 34, 152–156

PODPEČAN O., MRKUN J., ZRIMŠEK PETRA (2010): The evaluation of fat to protein ratio in milk as an indicator of calving to to conception interval in dairy cows using various biostatistical methods, Acta Veterinaria (Beograd), Vol. 60, No. 5-6, 541-550

Regulations on quality and other requirements for milk, dairy products, composite dairy products and starter cultures, Gazette SRJ No. 26/2002 and Gazette SCG No 56/2003

SILVA-DEL-RÍO N. , LAGO A., VERBOORT B., SELVARAJ H.(2011): Milk fat and milk protein to fat ratio in california dairies, university of california, agricultural and natural science, M282; ADSA, cetulare.ucdavis.edu/files/ 114681.pdf (12.01.2012).

TRBOJEVIĆ G.(1986): Osnovi biostatistike, Univerzitet u Beogradu, Veterinarski fakultet, Beograd

VERDIER-METZ I., COULON J.P, PRADEL P. (2001): Relationship between milk fat and protein contents and cheese yield, Anim.Res. 50, 365-371

VOS H., GROEN A.F. (1998): Altering milk protein/fat-ratio: results of a selection experiment in dairy cattle, Livestock Production Science Volume 53 (1), 49-55.

Received 25 September 2012; accepted for publication 20 December 2012

Biotechnology in Animal Husbandry 29 (1), p 161-171 , 2013
Publisher: Institute for Animal Husbandry, Belgrade-Zemun
ISSN 1450-9156
UDC 636.085:637.5'639.211
DOI: 10.2298/BAH1301161V

THE INFLUENCE OF NUTRITION ON RAINBOW TROUT (*ONCORHYNCHUS MYKISS*) MEAT QUALITY

D. Vranić[1], D. Trbović[1], J. Djinović-Stojanović[1], R. Baltić[2], M. Milijašević[1], S. Lilić[1], A. Spirić[1]

[1]Institute of Meat Hygiene and Technology, Kacanskog 13, 11000 Belgrade, Republic of Serbia
[2]Faculty of Veterinary Medicine, Bulevar oslobođenja 18, 11000 Belgrade, Republic of Serbia
Corresponding author: aurelija@inmesbgd.com
Communication

Abstract: Feed of high quality is one of the most important parameter which influences fish growth, feed conversion and chemical composition of fish meat in conditions of intensive fish farming. Studies on the influence of two commercial diets on nutritional quality and production results in rainbow trout intensive farming conditions in Pond I and Pond II were undertaken. Obtained values for proteins and fats in feed were significantly higher ($p<0.001$) in Diet II comparing to Diet I. The average water and ash content was significantly higher ($p<0.001$) in Diet I in relation to Diet II. The average length and mass of fish were higher in Pond I in relation to Pond II. Calculated values for condition factors, $K_{f,}$ indicate to a higher production results in Pond II (1.81) in relation to Pond I (1.22). There were not significant differences ($p>0.05$) in the average content of proteins and ash in fillets of fish from Pond I (17.43% and 1.19%, respectively) and Pond II (18.69% and 1.29%, respectively). The average content of water was significantly higher ($p<0.001$) in fillets of fish from Pond I (79.87%) comparing to fillets of fish from Pond II (75.40%). Average content of fat in fillets of trout from Pond II (4.17%) was around three times higher than the average content of fat in fillets of trout from Pond I (1.41%), ($p<0.001$). Results for energy values (348.44 kJ/100g- Pond I and 471.38 kJ/100g- Pond II) are in a direct correlation with the content of fat in fish. The average content of cholesterol in fillets of trout from Pond II (70.12 mg/100g) was significantly higher ($p<0.001$) than in fillets of trout from Pond I (44.11 mg/100g). The obtained data indicate to a positive influence of fat content in the diet on fat content, energy value and production results in rainbow trout production in Pond II. Lower water temperature established in Pond II (8°C) comparing to Pond I (13°C) might have had additional influence on the nutritional quality and production results in Pond II, as well.

Key words: rainbow trout, diet, proximate composition, production results, condition factor, cholesterol content.

Introduction

Nutritional and health benefits achieved by consumption of fish have changed populations' habits regarding fish consumption. That increased production and consumption of fish from aquaculture and imposed certain requirements to its' quality and nutritional value (*Burger and Gochfeld, 2009*). Low fat content, proteins of high biological value and relatively low cholesterol content as well as valuable quantities of essential fatty acids makes rainbow trout (*Oncorhynchus mykiss)* one of the most appreciable fish in human nutrition (*Conor, 2000; Sidhu, 2003*).

In conditions of intensive fish farming proper nutrition by offering suitable quantities of high-quality feed is the most important parameter which influences fish growth, feed conversion and chemical composition of fish meat *(Valente et al., 2007; Almeida et al., 2011*). In spite of that, salmonid fish species, where belong rainbow trout too, are the most demanding ones. Nutrition may affect, not only the ratio of proteins, fat and water, but the nutritional value of meat as well and the fatty acid composition (*Caballero et al., 2002).* However, nutritional quality of fish might be influenced by genetic factors, pH, oxygen content, water temperature and water quality, type of feed and feeding technology, etc. (*Skalli et al., 2006; Dube and Hosetti, 2010*).

Except highly digestible proteins and valuable fats fish contain cholesterol which might be unfavorable to human health. Literature data indicate that fish from aquaculture, although contain higher fat content than the same fish species from open waters, they contain similar quantities of cholesterol when expressed as g/100g fillets (*Cahu et al, 2004*). Other data indicate that cholesterol content in freshwater fish depends more on fish species than on type of rearing (*Moreira et al., 2001*). Considering clinical and epidemiological studies which point out to the connection between cholesterol in food, cholesterol in blood plasma and arteriosclerosis, relatively low content of cholesterol, makes trout a very suitable fish species for human consumption (*Orban et al., 2006*).

Annual consumption of fish in Serbia is around 7 kg per capita (*Baltic et al., 2009*). There are many reasons for such a low consumption, as leak in habits of eating fish, insufficient production, irregular market supply and orientation to import, week offer of diverse types of fish, high prices, leak of knowledge on consuming healthy food, etc. It is reliable that national production in aquaculture is insufficient and is mainly related to carp (*Cyprinus carpio*), over 80%, and rainbow trout (*Oncorhynchus mykiss*) production, around 15% (*Trbović et al., 2009; Vranić et al., 2011*). With the aim of contributing to development of aquaculture in the country, the objectives of this study were to investigate the influence of nutrition (two different commercial diets) on rainbow trout meat quality parameters

(proximate composition and cholesterol content) and production results in rainbow trout intensive farming conditions, as well.

Materials and Methods

Animals and sampling

Rainbow trout samples (*Oncorhynchus mykiss*) were collected during summer season from two intensive fish ponds (Pond I and Pond II) situated on different geographic locations. Fish ponds were supplied with water of potable quality directly from the springs. Water temperature in fish ponds was constant during the year, but different one in relation to other (Pond I-13°C, Pond II-8°C). Fish was manually fed commercially available extruded sinking pelleted diets (Diet I and Diet II) according to good aquaculture practice. Based on specifications, diet I consisted of fish meal (27.0%), chops after soybean extraction (22.0%), wheat (10.0%), soybean protein concentrate (7.8%), extruded soybean seed (7.0%), rapeseed chops (5.3%), rapeseed, fish and palm oils, corn gluten, amino acids by-products, organic weat, minerals and vitamins. Diet II consisted of fish products (60.0%), oils and fats (17.5%), products and by-products of weat (14.0%), products and by-products of oil seeds (6.5%) and vitamin premixes (2%). During sampling, six fishes were collected from each pond along with the appropriate feed (n=6). Fillets of fish, obtained after evisceration and previous deprivation of skin, tail, head, fins and bones were homogenized in a laboratory blender (Braun CombiMax 600), separately placed in plastic bags and stored at -25°C until analyzed. A day before analysis samples were defrosted overnight, at +4°C.

Chemicals and standards

Pro analysis quality and HPLC-grade solvents were obtained from Merck (Darmstadt, Germany) and Sigma Aldrich (Germany). All glassware was rinsed with acetone and hexane. Solvent blanks were analyzed with each lot of reagents.
More detailed data on proximate composition determination and saponification method for cholesterol determination by HPLC has been presented in our previous publication (*Spirić et al., 2009).*

A brief review of proximate composition (proteins, water, lipids and ash) and cholesterol determination is presented below.

Physical parameters of fish

After reception, mass of fish was measured on a technical balance, with ±0.01g accuracy. Fish length, from the tip of the head to the end of the tail, was measured by ruler, with ±0.1cm accuracy.

Condition factor (K_f) has been calculated from the average values for length and mass of fish by applying the following equation: $K_f=100 \cdot m \cdot l^{-3}$, where is: m-mass of the fish, in g and l-length of the fish, in cm.

Proximate composition (proteins, water, lipids and ash) determination

Proximate composition of fish fillets and feed was determined by ISO and in-house validated methods. Protein content was determined by Kjeldahl – N*6.25 (Kjeltec Auto 1030 Analyzer, Tecator, Sweeden). Water content was determined by drying at 103±2ºC to constant weight. For determination of total fat, samples were hydrolyzed with 4M hydrochloric acid and extracted with petroleum ether by Soxhlet apparatus. Ash was determined by combustion at 550±25ºC.

Cholesterol determination by HPLC

Cholesterol content was determined, after direct saponification of fish muscle at 80°C, by Waters 2695 HPLC/PDA system, on a Phenomenex Luna C18 reverse phase column, at 210 nm. Quantification was performed by external standardization in a linear concentration range from 25 mg/100 g to 125 mg/100 g. Recoveries of the spiked quantities ranged from 66.30 to 74.80%.

Statistical analysis

For data analysis UnscramblerX statistical software (CamoSoft, Norway) was used. Means within each group were compared with ANOVA and Tukey - Kramer's multiple range tests.

Results and Discussion

Proximate composition (protein, water, fat and ash) of commercial diets for trout breed in Pond I and Pond II is presented in Table 1. The obtained data indicate that the average values for proteins and fats were significantly higher, ($p<0.001$), in Diet II (44.81±0.11% and 25.33±0.09%, respectively) comparing to Diet I (42.60±0.04% and 18.08±0.04%, respectively). The average water and ash content was significantly higher, ($p<0.001$), in Diet I (7.41±0.02% and 8.18±0.14%, respectively) in relation to Diet II (6.40±0.03% and 4.87±0.05%, respectively). It was demonstrated that feed of high quality is one of the most important parameter which influences fish growth, feed conversion and chemical composition of fish meat in conditions of intensive fish farming *(Caballero et al., 2002; Valente et al., 2007*).

Table 1. Protein, water, fat and ash content (%, wet weight basis) in commercial diets for trout

Parameter	Pond	$\overline{X}$	Measures of dispersion				
			Sd	Se	Iv		CV (%)
					X_{min}	X_{max}	
Protein	I	42.60^{α}	0.04	0.02	42.55	42.65	0.09
	II	44.81^{β}	0.11	0.05	44.64	44.96	0.25
Water	I	7.41^{α}	0.02	0.01	7.39	7.44	0.26
	II	6.40^{β}	0.03	0.01	6.36	6.44	0.43
Fat	I	18.08^{α}	0.04	0.02	18.01	18.12	0.22
	II	25.33^{β}	0.09	0.04	25.21	25.42	0.34
Ash	I	8.18^{α}	0.14	0.06	8.02	8.32	1.67
	II	4.87^{β}	0.05	0.02	4.81	4.94	1.06

$^{\alpha, \beta}$ ($p<0,001$)

The obtained production results for trout breed in Pond I and II, feed two different commercial diets, are presented in Table 2.

Table 2. Production results for marketable trout

Parameter	Pond	$\overline{X}$	Measures of dispersion				
			Sd	Se	Iv		CV (%)
					X_{min}	X_{max}	
Lenght (cm)	I	28.22^{α}	0.66	0.27	27.30	29.00	2.36
	II	23.28^{β}	1.06	0.43	22.00	24.50	4.55
Mass of fish (g)	I	273.33	37.24	15.20	225.00	315.00	13.62
	II	228.83	41.38	16.89	170.00	290.00	18.08
Mass of fillets (g)	I	93.33	24.63	10.06	65.00	130.00	26.39
	II	97.26	18.22	7.44	65.57	121.41	18.73

$^{\alpha, \beta}$ ($p<0,001$)

As it can be seen from the presented results, the average length and mass of fish were higher in Pond I (28.22±0.66 cm and 273.33±37.24 g, respectively) in relation to Pond II (23.28±1.06 cm and 228.83±41.38 g, respectively). However, significant difference was established in relation to the average length of fish ($p<0.001$) only. Difference in the average mass of fish was not significant ($p>0.05$). The average mass of fillets in fish from Ponds I and II was 93.33±24.63 and 97.26±18.22 g, respectively and no significant difference was established ($p>0.05$). Even the average length and mass of fish were higher in Pond I in relation to Pond II, the obtained values for condition factors, K_f, (Table 3.) indicate to a higher production results in Pond II (1.81) in relation to Pond I (1.22). Higher production results in Pond II might be influenced by differences in types and share of feed ingredients, as established in the study conducted by *De Francesco et al. (2004)*. They demonstrated that higher K_f, lower mass and length was established in trout

fed feed containing higher share of maize, wheat and rapeseed meal comparing to trout predominantly fed feed containing fish meal.

Table 3. Condition factor (K_f) for marketable trout

Parameter	Pond	$\overline{X}$
Kondition faktor (K_f)	I	1.22
	II	1.81

Results for proximate composition (protein, water, fat and ash content) of marketable trout fillets are presented in Table 4.

Table 4. Protein, water, fat and ash content (%, wet weight basis) in marketable trout fillets

Parameter	Pond		Measures of dispersion				
		$\overline{X}$	Sd	Se	Iv		CV (%)
					X_{min}	X_{max}	
Protein	I	17.43	1.33	0.54	15.76	19.23	7.66
	II	18.69	0.46	0.19	17.95	19.32	2.48
Water	I	79.87^{α}	2.13	0.87	76.55	82.03	2.67
	II	75.40^{β}	1.14	0.46	74.38	77.36	1.51
Fat	I	1.41^{α}	0.70	0.29	0.63	2.45	49.75
	II	4.17^{β}	0.51	0.21	3.20	4.75	12.34
Ash	I	1.19	0.15	0.06	1.03	1.36	12.23
	II	1.29	0.02	0.01	1.26	1.31	1.39

$^{\alpha, \beta}$ ($p<0,001$)

The presented results indicate that there were not significant differences ($p>0.05$) in the average content of proteins and ash in fillets of marketable trout in Pond I (17.43±1.33% and 1.19±0.15%, respectively) and Pond II (18.69±0.46% and 1.29±0.02%, respectively). The average content of water was significantly higher ($p<0.001$) in fillets of fish from Pond I (79.87±2.13%) than in fillets of fish from Pond II (75.40±1.14%). The average fat content in fillets of trout in Pond II (4.17±0.51%) was around three times higher than the average fat content in fillets of trout in Pond I (1.41±0.70%), ($p<0.001$). It was established that fat content in fish is in a direct correlation with the nature and content of fat in the diet and that water content in fish flesh is inverse proportional to fat content (*Kaushik, 1995*). Fat content in marketable trout fillets was independent on the protein content in the diet (Pond I-42.60%, Pond II-44.81% proteins), what is in agreement with the data reported by *Lupatsch et al. (2001).* According to their results, fat content in feed had influence on the fat content in fish, while content of proteins in feed had no influence on protein content in fish. Protein content in salmonidaes is correlated with the size of fish and it is endogenously controlled, while their fat content is

influenced by numerous endogenous and exogenous factors (*Shearer, 1994).* Energy values for marketable trout fillets are presented in Table 5. The obtained results indicate that energy values for marketable trout fillets are statistically significantly higher ($p<0.001$) in fillets of fish from Pond II (471.38 kJ/100g) in relation to fillets of fish from Pond I (348.44 kJ/100g). These results are in a direct correlation with the content of fat in fish (1.41% fat in fillets of fish from Pond I and 4.17% fat in fillets of fish from Pond II). The increased energy value of feed increases the content of fat and decrease the content of water in fish (*Médale, 2010*).

Haliloglu and Aras (2002) and *Skalli et al. (2006)* demonstrated that nutritional quality of fish, except diet and other factors, might be influenced by water temperature as well. Our data confirm the positive influence of fat content in the diet on fat content and energy value of the fish. However, lower water temperature established in Pond II (8°C) comparing to Pond I (13°C) might have had some additional impact on fat content in muscle tissue of fish from Pond II, as well. *Martinez et al. (1992)* reported that temperature was the factor which had the strongest influence on fat and water content in body composition of rainbow trout.

Table 5. The energy value of marketable trout fillets

Energy value	Pond	$\overline{X}$	Measures of dispersion				
			Sd	Se	Iv		CV (%)
					X_{min}	X_{max}	
kJ/100 g	I	348.44^{α}	44.19	16.47	298.25	417.53	11.58
kJ/100 g	II	471.38^{β}	24.88	9.27	423.51	493.60	4.82

$^{\alpha, \beta}$ ($p<0,001$)

Results for cholesterol content in fillets of marketable trout are presented on Table 6. The average cholesterol content in fillets of marketable trout from Pond II (70.12 mg/100g) was significantly higher ($p<0.001$) than in fillets of fish from Pond I (44.11 mg/100g). Cholesterol content we established for *Oncorhynchus mykiss* is in accordance with the data obtained by *Kopicova and Vavreinova (2007),* of 41 mg/100g, for *Salmo trutta*, *Piironen et al. (2002),* of 60-65 mg/100g, for *Salmo garinderi* and *Nettleton and Exler (1992),* of 60 mg/100g, *for Oncorhynchus mykiss.*

Table 6. Cholesterol content (mg/100 g, wet weight basis) in fillets of marketable trout

Parameter	Pond	$\overline{X}$	Measures of dispersion				
			Sd	Se	Iv		CV (%)
					X_{min}	X_{max}	
Cholesterol (mg/100 g)	I	44.11^{α}	3.29	1.34	39.27	48.26	7.45
	II	70.12^{β}	14.25	5.82	44.40	84.08	20.32

$^{\alpha, \beta}$ ($p<0,001$)

De Francesco et al. (2004) has established that cholesterol content in trout was highly correlated with the content and type of feed ingredients. Other literature data indicate to a variability in cholesterol content, depending on genetic potential, season, age, gender, water temperature, etc (*Jhaveri et al., 1984).* Thus, it might be assumed that significantly higher content of cholesterol we obtained in fillets of trout in Pond II compared to Pond I might be influenced, not only by differences in type and share of feed ingredients, but by differences in water temperature, as well (Pond I-13°C, Pond II-8°C). According to *Krzynowek et al. (1982),* due to some physiological reasons, high body cholesterol was established in some fish species living in deep cold waters.

Conclusion

Based on the presented results, we can conclude that higher content of fat in the diet for fish in Pond II is reflected on higher content of fat in the fish fillets. However, due to the valuable fatty acids present in fatty component of fist and of some other nutrients as well, higher content of fat can be estimated as a significant parameter of fish quality and should not be considered as a negative influence of diet. Higher production results obtained in Pond II in relation to Pond I can be considered as a contribution of higher content of fat and higher energy value of the relevant diet. Consequently, higher content of cholesterol in fish from Pond II in relation to fish from Pond I might be a result not only of differences in type of feed ingredients and their share in the diet, but of the season, gender and/or water temperature as well.

Acknowledgement

This work was supported by grants from the Ministry of Education, Science and Technological Development; Republic of Serbia (projects No.TR20122 and TR31011).

Uticaj ishrane na kvalitet mesa kalifornijske pastrmke (Oncorhynchus mykiss)

D. Vranić, D. Trbović, J. Djinović-Stojanović, Ž. M. Baltić, M. Milijašević, S. Lilić, A. Spirić

Rezime

Hrana visokog kvaliteta je jedan od najvažnijih parametara koji utiču na rast ribe, konverziju hrane i hemijski sastav mesa ribe u uslovima intenzivnog uzgoja. Proučavan je uticaj dve vrste komercijalne hrane na nutritivni kvalitet i proizvodne rezultate u uslovima intenzivne proizvodnje kalifornijske pastrmke, u ribnjaku I i II. Dobijeni rezultati za proteine i masti u hrani bili su značajno veći ($p<0,001$) u hrani II u poređenju sa hranom I. Prosečan sadržaj vode i pepela bio je značajno veći ($p<0,001$) u hrani I u odnosu na hranu II. Prosečna dužina i masa ribe bila je veća u ribnjaku I u odnosu na ribnjak II. Izracunate vrednosti za kondicioni faktor, K_f, ukazuju na bolje proizvodne rezultate u ribnjaku II (1,81) u odnosu na ribnjak I (1,22). Nije bilo značajnih razlika ($p>0,05$) između prosečnog sadržaja proteina i pepela u filetima ribe iz ribnjaka I (17,43% i 1,19%, respektivno) i ribnjaka II (18,69% i 1,29%, respektivno). Prosečni sadržaj vode bio je značajno veći, ($p<0,001$), u filetima ribe iz ribnjaka I (79,87%) u odnosu na filete ribe iz ribnjaka II (75,40%). Prosečni sadržaj masti u filetima pastrmke iz ribnjaka II (4,17%) bio je oko tri puta veći od prosečnog sadržaja masti u filetima pastrmke iz ribnjaka I (1,41%), ($p<0,001$). Energetska vrednost (348,44 kJ/100g – ribnjak I i 471,38 kJ/100g – ribnjak II) je u direktnoj korelaciji sa sadržajem masti u ribi. Prosečni sadržaj holesterola u filetima pastrmke iz ribnjaka II (70,12 mg/100g) bio je značajno veći ($p<0,001$) u odnosu na sadržaj holesterola u filetima pastrmke iz ribnjaka I (44,11 mg/100g). Dobijeni rezultati ukazuju na pozitivan uticaj sadržaja masti iz hrane na sadržaj masti, energetsku vrednost i proizvodne rezultate kod kalifornijske pastrmke iz ribnjaka II. Takođe, i niža temperatura vode u ribnjaku II (8°C), u poređenju sa ribnjakom I (13°C), mogla je imati dodatni uticaj na bolji nutritivni kvalitet i proizvodne rezultate u ribnjaku II.

References

ALMEIDA I., MARTINS H.M., SANTOS S., FREITAS S.G., BERNARDO F. (2011): Mycobiota in feed for farmed sea bass (D*icentrarchus labrax*). Biotechnology in Animal Husbandry, 27, 93-100.

BALTIĆ Ž.M., KILIBARDA N., DIMITRIJEVIĆ N. (2009): Factors significant for the shelf-life of fish and selected fish products in retail. Tehnologija mesa, 50, 166-176.

BURGER J., GOCHFELD M. (2009): Perceptions of the risks and benefits of fish consumption: Individual choices to reduce risk and increase health benefits. Environmental Research, 109, 343–349.

CABALLERO M.J., OBACH A., ROSENLUND G., MONTERO D., GISVOLD M., IZQUIERDO M.S. (2002): Impact of different dietary lipid sources on growth,

lipid digestibility, tissue fatty acid composition and histology of rainbow trout, *Oncorhynchus mykiss*. Aquaculture, 214, 253-271.
CAHU C., SALEN P., DE LORGERIL M. (2004): Farmed and wild fish in the prevention of cardiovasular diseases: Assessing possible differences in lipid nutritional values. Nutrition Metabolism and Cardiovascular Diseases, 14, 34–41.
CONOR W.E. (2000): Importance of n-3 fatty acids in health and disease. American Journal of Clinical Nutrition, 71, 171S-175S.
DE FRANCESCO M., PARISI G., MEDALE F., LUPI P., KAUSHIK S., POLI B. (2004): Effect of long term feeding with a plant protein mixture based diet on growth and body/fillet quality traits of large rainbow trout (*Oncorhynchus mykiss).* Aquaculture, 236, 413-429.
DUBE P.N., HOSETTI B.B. (2010): Behaviour surveillance and oxygen consumption in the freshwater fish *labeo rohita* (hamilton) exposed to sodium cyanide. Biotechnology in Animal Husbandry, 26, 91-103.
HALILOĞLU H.I., ARAS N.M. (2002): Comparison of muscle fatty acids of three trout species (*Salvelinus alpinus, Salmo trutta fario, Oncorhynchus mykiss*) raised under the same conditions. Turkish Journal of Veterinary and Animal Sciences, 26, 1097–1102.
JHAVERI S.N., KARAKOLTSIDIS P.A., MONTECALVO J., CONSTANIDIS S.M. (1984): Chemical composition and protein quality of some southern New England marine species. Journal of Food Science, 49, 110-113.
KAUSHIK S.J. (1995): Nutrient requirements, supply and utilization in the contest of carp culture. Aquaculture, 129, 225-241.
KOPICOVA Z., VAVREINOVA S. (2007): Occurrence of squalene and cholesterol in various species of Czech freshwater fish. Czech Journal of Food Sciences, 25, 195-201.
KRZYNOWEK J., WIGGIN K., DONAHUE P. (1982): Cholesterol and fatty acid content in three species of crab in the northwest Atlantic. Journal of Food Science, 47, 1025-1026.
LUPATSCH I., KISSIL G.W., SKLAN D., (2001): Optimization of feeding regimes for European sea bass *Dicentrarchus labrax*:A factor approach. Aquaculture, 202, 289-302.
MARTINEZ F.J., GARCIA M.P., CANTERAS M., DE COSTA J., ZAMORA S. (1992): Effect of simultaneous variation of weight, density, temperature and O_2 concentration on rainbow trout (O. mykiss) body composition. Reproduction Nutrition Development, 32, 105-112.
MEDALE F. (2010): Nutrition quality of fish flesh lipids as affected by farming practices. Cahiers de nutrition et de diététique, 45, 267-273.
MOREIRA A.B., VISENTAINER J.V., DE SOUZA N.E., MATSUSHITA M. (2001): Fatty acids profile and cholesterol contents of three Brazilian *Brycon* freshwater fishes. Journal of Food Composition and Analysis, 14, 565–574.

NETTLETON J.A., EXLER J. (1992): Nutrients in wild and farmed fish and shellfish. Journal of Food Science, 57, 257-260.
ORBAN E., MASCI M., NEVIGATO T., DI LENA G., CASINI I., CAPRONI R., GAMBELLI L., DE ANGELIS P., RAMPACCI M. (2006): Nutritional quality and safety of whitefish (*Coregonus lavaretus*) from Italian lakes. Journal of Food Composition and Analysis, 19, 737–746.
PIIRONEN V., TOIVO J., LAMPI A.M. (2002): New data for cholesterol contents in meat, fish, milk, eggs and their products consumed in Finland. Journal of Food Composition and Analysis, 15, 705-713.
SHEARER K.D., (1994): Factors affecting the proximate composition of cultured fishes with emphasis on salmonids. Aquaculture, 119, 63-88.
SIDHU K.S. (2003): Health benefits and potential risks related to consumption of fish or fish oil. Regulatory Toxicology and Pharmacology, 38, 336–344.
SKALLI A., ROBIN J.H., LE BAYON N., LE DELLIOU H., PERSON-LE RUYET J. (2006): Impact of essential fatty acid deficiency and temperature on tissues' fatty acid composition of European sea bass (*Dicentrarchus labrax*). Aquaculture, 255, 223–232.
SPIRIĆ A., TRBOVIĆ D., VRANIĆ D., DJINOVIĆ J., PETRONIJEVIĆ R., MILIJASEVIĆ M., JANKOVIĆ S., RADICEVIĆ T. (2009): Fatty acid composition, cholesterol and total fat content in rainbow trout (*Oncorhynchus mykiss*) as influenced by fatty acids in diet. Tehnologija mesa, 50, 179-188. www.inmesbgd.com
TRBOVIĆ D. VRANIĆ D. DJINOVIĆ J. BOROVIĆ B. SPIRIĆ D. BABIĆ J., SPIRIĆ A. (2009): Fatty acid profile and cholesterol content in muscle tissue of one year old common carp (*Cyprinus carpio*) during growth. Tehnologija mesa, 50, 276-286. www.inmesbgd.com
VRANIĆ D., ĐINOVIĆ-STOJANOVIĆ J., SPIRIĆ A. (2011): Rainbow trout (*Oncorhynchus Mykiss*) from aquaculture – meat quality and importance in the diet. Tehnologija mesa, 52, 122-133. www.inmesbgd.com
VALENTE L.M.P., BANDARRA N. M., FIGUEIREDO-SILVA A.C., REMA P., VAZ-PIRES P., MARTINS S., PRATES J.A.M., NUNES M.L. (2007): Conjugated linoleic acid in diets for large-size rainbow trout (*Oncorhynchus mykiss*): effects on growth, chemical composition and sensory attributes. British Journal of Nutrition, 97, 289–297.
Received 25 September 2012; accepted for publication 20 December 2012

Biotechnology in Animal Husbandry 29 (1), p 173-181 , 2013 ISSN 1450-9156
Publisher: Institute for Animal Husbandry, Belgrade-Zemun UDC 633.853.52
DOI: 10.2298/BAH1301173A

THE EFFECT OF THE FIRST FERTILE FLOOR ON QUALITATIVE – QUANTITATIVE PROPERTIES OF SOYBEAN SEED

S. Anđelović[1], S. Maksimovic[2], D. Savić[3], Z. Tomic[4], D. Delić[2]

[1]Delta Agrar doo Belgrade, Serbia
[2]Institute of Soil Science, Belgrade, Serbia
[3]University of Belgrade, Faculty of Agriculture, Zemun, Serbia
[4]Institute of Animal Husbandry, Belgrade, Serbia
Corresponding author: srdjan.andjelovic@deltaagrar.rs
Communication

Abstract: The aim of this study was to determine the effect of height of the first fertile floor on the qualitative and quantitative characteristics of soybean seeds. For qualitative traits the focus was on the energy of germination and seed germination of the studied genotypes investigated per soybean plant and the first fertile floor of the plant. Results of research indicated that there were significant differences between genotypes observed by morphological characteristics (plant height, height of first fertile floor, number of fertile floors, number of pods and seeds, seed weight, etc.) as well as qualitative properties (energy of germination and seed germination). Cultivar Gorštak, with genetically incorporated height of the first fertile floor (12.38 cm), was superior to other two genotypes. A similar trend was found in other morphological analyses. Based on energy of germination and seed germination of all fertile floors per plant, there were no significant differences between soybean genotypes. However, of paramount importance are the established values of these parameters relevant to the first fertile floor. Cultivar Gorštak had significantly higher energy of germination (90.46%) and total germination (91.00%) compared to the other two genotypes.

Key words: soybean, genotype, seed quality, germination, yield

Introduction

The soybean (*Glycine max*) is annual legume widely grown for its edible bean which has numerous uses. The plant is classed as an oilseed rather than a pulse. Soybean genomics is of great interest as one of the most economically important crops and a major food source. Most soybeans are processed for their oil and protein for the animal feed industry. Protein content accounts for about 40% of dry soybeans while carbohydrates and oils account for about 35% and 20%,

respectively. Because soybeans have high protein content, they are a major ingredient in livestock feed. A smaller percentage is processed for human consumption and made into products including soy milk, soy flour, soy protein, tofu and many retail food products. Soybeans are also used in many non-food (industrial) products. Recently, soybean oil has caused considerable attention due to its increased use for biodiesel production. High quality soybeans are grown, harvested and purchased by the seed industry to be used as seed for the next year's crop. Researchers in the seed industry focus on developing new soybean varieties with outstanding characteristics including high yield, lodging resistance, nematode resistance, herbicide tolerance, and many other desirable characteristics (*Chung et al., 2003; Scott et al., 1970; Aćimović, 1988*). Height of first fertile floor is of very practical importance in the production of soybeans, especially in seed production. In conditions of low first fertile floor (<10 cm) crop losses increased by over 5% with a significant reduction of seed quality (energy of germination, germination and health). Particularly aggravating circumstance in seed crops with low first fertile floor is poor quality of seeds from lower floors which get into the total amount of processed and dressed seeds causing decrease of seed quality.

Materials and Methods

Two cultivars (Tijana and Gorstak) and one homozygous soybean line (BG-L-4601) with different levels of height of first fertile floor were examined (Table 1). Cultivars Tijana, BG-L-4601 and Gorstak belonged to 0, I and II maturity group, respectively. The experiment was carried out in 2011 at the experimental field of AD Progress, Stara Pazova, in completely randomized design with four replicates. The cultivars had the same sowing and germination date. The harvest date was 9. 15. and 24. September for Tijana, BG-L-4601 and Gorstak, respectively. Number of plants in harvest ha^{-1} were 480 000, 430 000 380 000 for Tijana, BG-L-4601 and Gorstak, respectively. Experimental plot was 5 m x 2 m with 50 cm distance row-to-row. Each plot was planted as four rows of 5 m length. During the growing season, plant phenological and phyto-pathological observations were carried out. After the harvest, morphological analysis of plants from two inner rows was done. Also, quality of seed (energy of germination, seed germination and seed health especially, on the first fertile floor) was determined. All data were subject to ANOVA using the statistical analysis system (SPSS, 2007. SYSTAT version 16: Statistics. SPSS Chicago, IL, USA.). Means of all parameters were calculated and the differences tested for significance using the LSD test at the 0.5 probability level. Correlation coefficients were calculated to study the associative relations among the measured traits.

Results and Discussion

Sowing was performed at optimal time with a homogeneous germination of plants which provide a high quality of used seed in all three genotypes. Harvesting is done manually after achievement of physiological maturity of certain genotypes. Morphological analysis of plants related to plant height showed a high degree of significance of differences ($P<0.05$) between all three genotypes (Table 1). Cultivar Gorštak was superior (110.83 cm), while the differences between cultivar Tijana (98.15 cm) and line BG-4601-L (90.73 cm) were also highly significant.

Table 1. Plant height, height of the first fertile floor and number of first floor of soybean cultivars, line

Replication	Cultivar, line								
	Plant height (cm)			The height of the first fertile floor (cm)			The number of first floor (cm)		
	Tijana	BG-L-4601	Gorštak	Tijana	BG-L-4601	Gorštak	Tijana	BG-L-4601	Gorštak
I	98,10	92,15	110,10	10,25	7,00	12,75	14,05	13,75	18,90
II	97,00	89,00	112,00	11,00	7,15	12,60	14,00	13,60	18,80
III	99,00	90,00	110,05	10,00	6,90	12,05	13,95	14,00	19,15
IV	98,50	91,75	111,15	9,85	6,85	12,10	14,10	13,55	18,95
X	98,15	90,73	110,83	10,28	6,98	12,38	14,03	13,73	18,95
	LSD 0,05=1,798			LSD 0,05=0,586			LSD 0,05=0,238		

Similar trends have been shown related to the height of first fertile floor (Table 1). Cultivar Gorštak (12.38 cm) had significantly higher height of the first fertile floor compared to other two genotypes, but also significant differences were found between cultivar Tijana (10.28 cm) and line BG-L-4601 (6.98 cm). Height of the first fertile floor is under very important influence of genetic factors which is confirmed by other researchers *(Plazinić, 1987; Plazinić et al., 1999)*. Number of fertile floors per plant was very significantly higher in the cultivar Gorštak (18.95) than the other two genotypes, while the differences between cultivar Tijana (14.03) and the line BG-L-4601 (13.73) were also, significant (Table 1).

The number of pods per plant in the cultivar Gorštak (48.09) was significantly higher than in other two genotypes (40.46 - Tijana, 40.16 - line BG-L-4601), while the differences between these two genotypes were also significant (Table 2). The number of pods per plant generally is higher in the dominant genotypes *(Sincler and Shurtleft, 1975; Scott and Aldrich, 1970*). The number of seeds per plant followed the trend of number of pods per plant. Highly significant differences between cultivar Gorštak (115.41) and the other two genotypes were

established, while there were no significant differences between the cultivar Tijana (96.89) and the line BG-L-4601 (97.23) (Table 2). Similar results have been reported by *Andjelović et al. (2000)* and *Plazinić (1987)* indicating a significant correlation between the number of pods and seeds. The number of pods on the first fertile floor of Tijana and BG-L-4601 (3.98 - Tijana, 4.08 - BG-L-4601) was significantly higher in relation to the cultivar Gorštak (3.01), while the there was no difference between the first two mentioned genotypes (Table 2). The temperature had significant effect on the number pods and seeds per plant (*Wigham and Minor, 1978*)

Table 2. **The number of pods and seeds per plant and the number of pods on the first fertile floor of soybean cultivars, line**

Replication	Cultivar, line								
	The number of pods per plant			The number of seeds per plant			The number of pods on the first fertile floor		
	Tijana	BG-L-4601	Gorštak	Tijana	BG-L-4601	Gorštak	Tijana	BG-L-4601	Gorštak
I	41,00	40,00	48,15	99,22	98,00	115,56	4,15	4,10	3,10
II	40,10	39,75	47,95	96,44	96,75	115,65	3,90	4,05	3,00
III	40,75	39,80	48,00	96,00	95,52	114,83	3,85	4,00	2,95
IV	40,00	41,10	48,25	95,90	98,64	115,60	4,00	4,15	3,00
X	40,46	40,16	48,09	96,89	97,23	115,41	3,98	4,08	3,01
				LSD 0,05=1,966			LSD 0,05=0,148		

Number of seeds on the first floor of the first two genotypes (9.38 - Tijana, 9.55 - BG-L-4601) (Table 3) was very significantly higher compared to cultivar Gorštak (5.34), whereas differences between the first two genotypes were not statistically significant. The mass of seeds per plant was very significantly different between the genotypes (Table 3). Cultivar Gorštak (19.15 g) was highly significantly superior in regard to these quantitative traits in relation to the other two genotypes. At the same time, Tijana (15.53 g) had significantly greater mass of seeds in relation to the line BG-L-4601 (13.83 g). Differences in seed weight per plant were formed as a result of a domination of genetic factors and the interaction between genotype and agricultural conditions. Similar results have been reported by *Anđelović et al. (2000)*. Seed weight on the first fertile floor differed significantly between the three genotypes (Table 3). Cultivar Gorštak (0.80 g) had significantly lower seed weight than the other two genotypes, whereas no significant differences were established between them (1.69 g - Tijana, 1.60 g - BG-L-4601).

Energy of germination and seed germination are very important qualitative properties and their practical values directly or indirectly influence the production of seeds and grains in field conditions.

Table 3. The number of seeds and seed weight on the first fertile floor of soybean, seed weight per plant of a soybean cultivars, line

Replication	Cultivar, line								
	The number of seeds on the first fertile floor			Seed weight per plant (g)			Seed weight on the first fertile floor (g)		
	Tijana	BG-L-4601	Gorštak	Tijana	BG-L-4601	Gorštak	Tijana	BG-L-4601	Gorštak
I	9,96	9,63	5,52	16,01	14,01	19,10	1,80	1,60	0,83
II	9,00	9,56	5,46	15,40	13,68	19,95	1,62	1,57	0,82
III	8,95	9,35	5,00	15,62	13,50	18,80	1,62	1,62	0,75
IV	9,60	9,65	5,36	15,10	14,11	18,76	1,73	1,61	0,81
X	9,38	9,55	5,34	15,53	13,83	19,15	1,69	1,60	0,80
	LSD 0,05=0,515			LSD 0,05=0,701			LSD 0,05=0,090		

Energy of seed germination of all fertile floors showed no significant differences between genotypes (Table 4). Both cultivars - Tijana 90.58% and 91.15% as Cultivar Gorštak and the line BG-L-4601 (90.56%) had high values for this parameter as qualitative property of seed. Seed germination of all fertile floors followed the energy of germination without significant differences between genotypes (91.88% Tijana, 91.50% BG-L-4601, 91.80% Cultivar Gorštak) (Table 4).

Table 4. Seed germination energy and seed germination of all fertile floors of soybean

Replication	Cultivar, line					
	Seed germination energy of all fertile floor (%)			Seed germination of all fertile floors (%)		
	Tijana	BG-L-4601	Gorštak	Tijana	BG-L-4601	Gorštak
I	89,70	90,35	92,00	92,00	91,50	92,00
II	91,00	91,20	91,70	92,50	92,00	92,50
III	90,10	89,95	90,90	91,00	91,00	91,70
IV	91,50	90,75	90,00	92,00	91,50	91,00
X	90,58	90,56	91,15	91,88	91,50	91,80
	LSD 0,05=1,227			LSD 0,05=0,903		

Energy of seed germination on the first fertile floor was significantly different between all genotypes (Table 5). Very significantly higher values of the qualitative properties of seeds were determined in the cultivar Gorštak (90.46%) compared to the other two genotypes. At the same time there was highly significant difference between cultivar Tijana (82.80%) and line BG-L-4601 (66.84%).

Differences between the genotypes showed very significant participation of genetic factors, which has been reported in other studies *(Plazinić, 1987; Brim, 1973.)*. Seed germination on the first fertile floor differed significantly between genotypes (Table 5). Very significant differences were observed between cultivars Gorštak (91.00%) and other two genotypes. At the same time, also, a significant difference between the qualitative characteristics of the cultivar Tijana (84.45%) and line BG-L-4601 (66.98%) was established.

Table 5. Energy of seed germination and seed germination of the first fertile floor of soybean

Replication	Cultivar, line					
	Energy of seed germination of the first fertile floor (%)			Seed germination of the first fertile floor (%)		
	Tijana	BG-L-4601	Gorštak	Tijana	BG-L-4601	Gorštak
I	82,80	68,00	90,00	84,00	68,00	91,50
II	83,00	66,35	90,75	85,10	66,55	91,00
III	83,50	66,56	91,00	85,00	66,00	91,00
IV	81,90	67,00	90,10	83,70	67,35	90,50
X	82,80	66,84	90,46	84,45	66,98	91,00
	LSD 0,05=1,115			LSD 0,05=1,107		

Correlations between investigated parameters are presented in Table 6. Plant height showed a high degree of significance of correlation with all examined traits except germination and seed germination of all fertile floors. Height of first fertile floor was positively correlated with the number of fertile floors (r = 0,813) number of pods (r = 0,799) and seeds (r = 0,774) per plant and the energy (r = 0,986) and germination (r = 0,983) of seeds on the first fertile floor. At the same time, it was negatively correlated with number of pods (r = -0,823) and seeds (r = -0,801) on the first fertile floor. The number of fertile floors was positively correlated with number of pods (r = 0,990) and seeds (r = 0,989) per plant, seed weight per plant (r = 0,944), and negatively correlated with number of pods (r = -0,986) and seeds (r = -0,990) as well as the mass of native seeds on the first fertile floor (r = -0,980). The number of pods on the first fertile floor was negatively correlated with plant height (r = -0,936), height of first fertile floor (r = -0,823), number of fertile floor per plant (r = -0,986) and the number of pods (r = -0,987) and seeds per plant (r = -0,957).

Number of seeds on the first fertile floor was negatively correlated with plant height (r = -0,924), height of first fertile floor (r = -0,801), the number of fertile floors (r = -0,990) and number of pods (r = -0,979) and seeds per plant (r = -0,969) and positively correlated with number of pods on the first fertile floor (r =0,997). Seed weight per plant was positively correlated with plant height (r =

0,988), height of first fertile floor (r = 0,930), the number of fertile floors (r = 0,944), number of pods (r = 0,948) and seeds per plant (r =0,937) and negatively correlated with number of pods (r = -0,933) and seeds (r = -0,926) on the first fertile floor.

Table 6. Correlations between investigated parameters of some soybean cultivars, line

[a] Parameters	V cm	VE cm	BETŽ	BM	BS	BMŽ1	BSŽ1	QS g	QSŽ1 g	EK %	UKS %	EKŽ1 %	UKSŽ1 %
V	1	0.939***	0.939***	0.936***	0.917***	-.0.936***	-0.924***	0.988***	-0.877***	0.335^{ns}	0.175^{ns}	0.935***	0.913***
VE		1	0.813**	0.799**	0.774**	-0.823***	-0.801**	0.930***	-0.725**	0.358^{ns}	0.349^{ns}	0.986***	0.983***
BETŽ			1	0.990***	0.989***	-0.986***	-0.990***	0.944***	-0.980***	0.36^{2ns}	0.099^{ns}	0.779**	0.742**
BM				1	0.995***	-0.970***	-0.979***	0.948***	-0.975***	0.341^{ns}	0.082^{ns}	0.767**	0.730**
BS					1	-0.957***	-0.969***	0.937***	-0.973***	0.356^{ns}	0.123^{ns}	0.734**	0.695*
BMŽ1						1	0.997***	-0.933***	0.980***	-0.367^{ns}	-0.080^{ns}	-0.792**	-0.757**
BSŽ1							1	-0.926***	0.990***	-0.368^{ns}	-0.073^{ns}	-0.766**	-0.729**
QS								1	-0.888***	0.361^{ns}	0.250^{ns}	0.910***	0.885***
QSŽ1									1	-0.390^{ns}	-0.062^{ns}	-0.680*	-0.638*
EK										1	0.674*	0.277^{ns}	0.285^{ns}
UKS											1	0.272^{ns}	0.291^{ns}
EKŽ1												1	0.998***
UKSŽ1													1

*,**,***Significant at P<0.05, 0.01, 0.001;

1. V Height of plant 2. VE Height of the first fertile floor (sm) 3. BETŽ Number of fertile floor 4. BM Number of pods 5. BS Number of seeds 6. BMŽ1 Number of pods on the first fertile floor 7. BSŽ1 Number of seeds on the first fertile floor 8. QS 1000 seeds weight per plant 9. QSŽ1 1000 seeds weight on the first fertile floor 10. EK Energy of seed germination 11. UKS Seed germination 12. EKŽ1 Energy of seed germination on the first fertile floor 13. UKSŽ1 Seed germination on the first fertile floor.

Correlations between energy and seed germination per plant, on the one hand, and plant height, height of first fertile floor, the number of fertile floors, number of pods and seeds per plant, number of pods and seeds on the first fertile floor, seed weight per plant and seed weight on the first fertile floor, on the other hand were not statistically significant. Energy of seed germination on the first fertile floor was positively correlated with plant height (r =0,935), height of first fertile floor (r =0,986), the number of fertile floors (r = 0,779), number of pods (r =0,767) and seeds per plant (r =0,734) as well as seed weight per plant (r =0,910). It also showed a negative correlation with number of pods (r = -0,792) and seeds on the first fertile floor (r = -0,766) and seed weight on the first fertile floor (r = -0,680). Seed germination on the first fertile floor was positively correlated with

plant height (r =0,913), height of first fertile floor (r =0,983), the number of fertile floors (r = 0,742), number of pods (r =0,730) and seeds per plant (r =0,695) as well as seed weight per plant (r =0,885) and negative correlation with number of pods (r = -0,757) and seeds on the first fertile floor (r = -0,729) and seed weight on the first fertile floor (r = -0,638).

Uticaj prve rodne etaže na kvalitativno –kvantitativna svojstva semena soje

S. Anđelović, S. Maksimovic, D. Savić, Z. Tomic, D. Delić

Rezime

Cilj istraživanja bio je utvrđivanje uticaja visine PRVE rodne etaže na kvalitativno-kvantitativna svojstva semena soje. Kod kvalitativnih svojstava akcenat je stavljen na energiju klijanja i ukupnu klijavost semena istraživanih genotipova posebno po biljci i prvoj rodnoj etaži. Sumiranjem rezultata istraživanja, utvrđene su značajne razlike između genotipova, posmatrano po morfološkim karakteristikama (visina biljaka, visina prve rodne etaže, broj rodnih etaža, broj mahuna i semena, masa semena i dr) kao i kod kvalitativnih svojstava – energije klijanja i ukupna klijavost semena. Sorta Gorštak sa genetički inkorporiranom visokom prvom rodnom etažom (12,38 cm) bila je dominantna u odnosu na druga dva genotipa. Sličan trend je utvrđen i kod drugih morfoloških analiza. Energija klijanja i ukupna klijavost semena svih rodnih etaža, posmatrano po genotipovima, nije pokazala značajne razlike. Međutim, od izuzetnog značaja su utvrđene vrednosti ovih parametara kada je reč o prvoj rodnoj etaži. Sorta Gorštak imala je vrlo značajnu veću vrednost energije klijanja (90,46%) kao i ukupnu klijavost semena (91,00 %) u odnosu na druga dva genotipa.

References

ANĐELOVIĆ S., PLAZINIĆ V., MARIĆ M., JOVANOVIĆ B. (2000): Vegetation area, yield components and seed quality. Collection of scientific works, INI Agroekonomik, Belgrade, 6, 117-121.

AĆIMOVIĆ M. (1988): The agents of soybean diseases and their control. Scientific Book, Belgrade, 69-80.

BRIM C.A. (1973): Soybeans:-Improvement, Production and Uses, Quantitative Genetics and Breeding, Madison, Wisconsin, Chapter 5, 155-183,

CHUNG J., BARKA STASWICK H.L. P.E., LEE D.J., GREGAN P.B., SHOEMAKER R.C., SPECHT J.E. (2003): The seed protein, oil and yield QTL on soybean linkage group I. Crop Sci., 43, 1053-1067.
PLAZINIĆ V. (1987): Contribution to the influence of genotypic and phenotypic variation in soybean seed. Archives of Agricultural Sciences, Belgrade, 48-68.
PLAZINIĆ V., ANDJELOVIĆ S., NENADIĆ N. (1999): Inheritance of first fertile floor in the new BG-lines. Collection of scientific works, INI Agroekonomik, Belgrade, 183-190.
SCOTT O.W., ALDRICH S.R. (1970): Modern Soybean Production. Champaign J., USA 11,.
SINCLAIR J.B., SHURTLEFF M.C. (1975): Compendium of Soybean Diseases. The American Phytopatological Society, St. Paul, Minn., USA.
WHIGHAM D.K., MINOR H.C.(1978): Effect of Temperature on Growth and Development. Soybean Biology, Agronomy and Utilization. Academic Press inc London.

Received 20 February 2013; accepted for publication 21 March 2013

Instruction for authors

Papers for publishing in the *Biotechnology in Animal Husbandry* journal should be submitted to the Editorial Board. Address: Institute for Animal Husbandry, Autoput 16, 11080 Belgrade-Zemun, P.O.box 23, Republic of Serbia (for *Biotechnology in Animal Husbandry*).

Original papers in English, (on a CD-ROM or by e-mail: biotechnology.izs@gmail.com) 6 pages of typed text using, Paper size: Custom size, Width 17 cm, Height 24 cm; formata (Portrait), normal spacing (Single Space). Margine: Top 2,0 cm, Left 2.0 cm, Bottom 2.0 cm, Right 2,0 cm, no pagination. Use font Times New Roman, size 11 (except where it is stated otherwise). Title of the paper should be Times New Roman, font size 14, **bold**:

Example 1

TABLE EGGS OF KNOWN ORIGIN AND GUARANTEED QUALITY - BRAND EGG

Authors, Times New Roman, font size 12, **bold**

Z. Pavlovski, Z. □krbić, M. Lukić

Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080, Belgrade-Zemun, Republic of Serbia
Corresponding author: zlaticapav@yahoo.com
Invited paper

Example 2

THE EFFECT OF PARAGENETIC FACTORS ON REPRODUCTIVE TRAITS OF SIMMENTAL COWS

M. D. Petrović[1], Z. Skalicki[2], V. Bogdanović[2], M. M. Petrović[3]

[1]Faculty of Agronomy, Cara Du□ana 34, 32000, Čačak, Republic of Serbia
[2]Faculty of Agriculture, Nemanjina 6, 11080, Belgrade-Zemun, Republic of Serbia
[3]Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080, Belgrade-Zemun, Republic of Serbia
Corresponding author: petrovicm@tfc.kg.ac.yu
Original scientific paper

use [1,2, ...] numbers in suffix to refer to addresses of authors, under affilations of authors should be mentioned e-mail of corresponding author and category of paper, Times New Roman, font size 9

Original scientific paper should contain following paragraphs with single spacing (title of paragraphs should be in Times New Roman 14 **bold,** except for **Abstract** and **Key words** where font size is 11 **bold**):

Abstract: 250 words

Key words: state key words (not more than 6)

Introduction - present the review of previous research and objective of the paper.

Materials and Methods - state methods applied in the paper.

Results and Discussion - present investigation results separately from discussion or together in one paragraph. Presentation of the results should be precise and without repetitions, and include the evaluation of significant differences and other parameters.

Text and titles of tables, figures and graphs, Times New Roman, font size 9, **bold**, in the following form:

Table 1. Least square means for the reproductive traits of cows

Tables and figures should be numbered and with adequate title and legend, width and height not exceeding 12 cm and 17 cm, respectively. Tables should be prepared according to instruction for forming of tables in Office Word. Each column in table must have heading and, when necessary, abbreviations should be explained in the legend/footnote.

Conclusion - containing the most important issues of the paper

Acknowledgment - for example:

Research was financed by the Ministry of Science and Technological Development, Republic of Serbia, project TR 6885.

After Acknowledgment the title of the paper in Serbian in Times New Roman 14 **bold**, is stated, followed by authors in Times New Roman 11 *italic*, example:

Konzumna jaja poznatog porekla i garantovanog kvaliteta - brand jaja

Z. Pavlovski, Z. krbić, M. Lukić

Summary - should contain the most important issues of the paper. It should be in English, and Serbian for domestic authors (min. 250 words).

References - should be in alphabetical order. Names of the authors must be given in capital letters followed by the year of publicationin brackerts, titles in the language of the original, examples:

PAVLOVSKI Z. (2004): Novi propisi EU, dobrobit ivine, zahtevi potroača. ivinarstvo, 8-9, 49-58.

PAVLOVSKI Z., MAIĆ B. (1994): Odnos potroača prema ivinskim proizvodima. ivinarstvo, 7-9, 77-82.

PETROVIĆ D.M., GUTIĆ M., BOGOSAVLJEVIĆ-BO□KOVIĆ S. (2004): Masa teladi pri rođenju i njena varijabilnost kod krava simentalske rase. Agroznanje, 5, 1, 111-116.

Citations in the text are presented in *italic* form, examples: ...results of *Pavlovski (2004)...*; *(Pavlovski and Ma□ić, 1994)*; *(Petrović et al., 2004)*; *(Pavlovski, 2004; Pavlovski and Ma□ić, 1994; Petrović et al., 2004)*.

Authors are fully responsible for the contents of the papers.

Biotechnology in Animal Husbandry contains three categories of papers:

- Original scientific paper,
- Review paper, and
- Communication.

Review papers must have minimum 5 self-citations (by the first author).

All papers are published in English, and reviewed.

Abbreviation for journal *Biotechnology in Animal Husbandry* is:
Biotechnol Anim Husb

Editorial Staff

Institute for Animal Husbandry, Belgrade-Zemun
10th International Symposium
"Modern Trends in Livestock Production"
2nd – 4th October 2013, Belgrade, Serbia

Hotel Park, Belgrade, Serbia

SECOND COMMUNICATION

The tenth International Symposium **"Modern Trends in Livestock Production"** will be organized from October 2-4 in Hotel Park, Belgrade, Serbia, in organization of Institute for Animal Husbandry, Belgrade-Zemun.

Full papers prepared for publication according to Instructions for authors for journal *Biotechnology in Animal Husbandry* (www.istocar.bg.ac.rs), should be submitted before **May 31st, 2013.** (Papers not prepared according to Instruction for authors will not be considered). Authors, kindly, state the method of presentation of the paper oral or poster.

Papers in English should be submitted to following e-mail address:
biotechnology.izs@gmail.com

or on CD, to the following address:
Institute for Animal Husbandry,
Autoput 16, P. Box 23,
11080 Belgrade-Zemun,
Serbia

For poster session - size of posters 60 x 90 cm (width x height).

Official language
Official language of Symposium is **English**. All oral presentations shall be interpreted.

Registration fee

Registration fee is obligatory for all Symposium participants except participants with invited plenary presentations.

- Registration fee which includes: publishing of paper in journal *Biotechnology in Animal Husbandry*, Symposium material, participation in all sessions of the Symposium, coffe/tea break, is **100 EUR** (for domestic participants in dinar value on the day of payment according to the exchange rate). Papers shall not be published without the payment of registration fee.
- Registration fee which includes: publishing of paper in journal *Biotechnology in Animal Husbandry*, Symposium material, participation in all sessions of the Symposium, coffe/tea break,cocktail, tourist programme and gala dinner, is **150 EUR** (for domestic participants in dinar value on the day of payment according to the exchange rate).
- **Deadline for payment of registration fee June 30th 2013**.

If authors need proforma invoice they can contact us by phone, +381 11 2670121, extensions 220, 203 (Vesna Krnjaja or Olga Devečerski) or e-mail address: biotechnology.izs@gmail.com

All papers presented (oral and posters) will be published in journal *Biotechnology in Animal Husbandry* (with two reviews)

Payment of registration fee before stated deadline is to be made on the following account :

INSTRUCTION FOR EUR PAYMENTS
AIK BANKA AD NIS

Please pay as pre instruction given below:

56A: Intermediary bank:	**COBADEFF** **COMMERZBANK AG** **60301 FRANKFURT AM MAIN** **GERMANY**
57A: Account with institution:	**AIKBRS22** **Acc.No. 400876864000EUR** **AIK BANKA AD** **NIKOLE PASICA 42** **18000 NIS** **SERBIA**
59: Beneficiary customer:	**INSTITUT ZA STOCARSTVO** **ZEMUN** **Autoput Beograd-Zagreb 16** **Zemun** **IBAN: RS35105050120000062319**

Symposium location and accommodation

Symposium will be held in **Hotel Park, Belgrade** Njego□eva street 2, 11000, Belgrade, Serbia

- Symposium participants can book accommodation at significantly lower prices :

Single room (bed and breakfast) **50 EUR**
Double room (bed and breakfast, 2 persons) **70 EUR**

- Tourist tax is not included in the price.

Deadline for accommodation booking at lower prices is **31st August 2013**

Address: Hotel Park, Njego□eva 2, 11000 Beograd
Tel. +381 11 3640385; +381 11 3640393
Fax. +381 11 3640393

E mail: **reception@hotelparkbeograd.rs**
www.hotelparkbeograd.rs

Chairman
Organizing Committee

Dr Milo□Lukić
Serbia

Chairman
International Scientific Committee

Prof.dr Martin Waehner
Germany

Институт за сточарство, Београд-Земун
10. међународни симпозијум о сточарству
Савремени трендови у сточарству
2.-4. октобар 2013. године, Београд, Србија

Хотел Парк, Београд, Србија

ДРУГО ОБАВЕШТЕЊЕ

Десети међународни симпозијум о сточарству **Савремени трендови у сточарству** одржаће се од 2. до 4. октобра 2013. године у Хотелу Парк, у Београду, Србија, у организацији Института за сточарство, Београд-Земун.

Радове, припремљене за штампу према упутству часописа *Biotechnology in Animal Husbandry* (www.istocar.bg.ac.rs), послати до **31. маја 2013.** године. (Радови који нису припремљени према упутству, биће враћени на измену или неће бити разматрани). Аутори би требало да наведу начин презентације рада, усмено предавање или постер.

Радове послати на енглеском језику на CD-у, на e-mail адресу:
biotechnology.izs@gmail.com

или поштом на адресу**:**

Институт за сточарство,
Аутопут 16, П. фах 23,
Земун-Београд 11080,
Србија

За секцију постера прилози се припремају у величини 60 x 90 cm (ширина x висина).
Службени језик
Службени језик симпозијума је **енглески**. Сва усмена излагања се симултано преводе.

Котизација

Котизацију уплаћују сви учесници симпозијума изузев учесника са пленарним предавањем

- Котизација која укључује: штампање рада у часопису *Biotechnology in Animal Husbandry*, материјал симпозијума, учествовање у свим секцијама симпозијума, кафу/чај у паузама, износи **100 EUR** (за домаће учеснике у динарима на дан уплате по важећем средњем курсу). Ниједан рад неће бити штампан без уплаћене котизације.
- Котизација која укључује: штампање рада у часопису *Biotechnology in Animal Husbandry*, материјал симпозијума, учествовање у свим секцијама симпозијума, кафу/чај у паузама, коктел, туристички програм и свечану вечеру, износи **150 EUR**.

  Рок за уплату котизација је **30. јун 2013.** године.

Уколико Вам је потребна профактура за уплату котизације можете нам се обратити телефоном, централа 011 2670 121, локали 220, 203 (Весна Крњаја или Олга Девечерски) или на e-mail адресу: biotechnology.izs@gmail.com

Међународни научни комитет симпозијума ће након пријема радова извршити две рецензије по раду. Рецензирани и прихваћени радови за штампу биће штампани у целости на енглеском језику у часопису *Biotechnology in Animal Husbandry*.

Уплату котизације потребно је у наведеном року извршити на рачун:

Институт за сточарство, Београд-Земун
11080 Земун, Аутопут 16
Комерцијална банка
Рачун бр. 205-65958-94

Место одржавања симпозијума и смештај

Симпозијум ће се одржати у **Хотелу Парк**, Његошева 2, 11000, Београд, Србија.
За учеснике симпозијума резервација смештаја до **31. августа 2013.** године је по знатно нижим ценама:

Једнокреветна соба (ноћење са доручком) **50 EUR**
Двокреветна соба (ноћење са доручком) **70 EUR**

Цена боравишне таксе није укључена у наведене цене.

Адреса: Хотел Парк, Његошева 2, 11000 Београд
Тел. 011/3640-385; 011/3640-393
Фах. 011/3640-393

E mail: **reception@hotelparkbeograd.rs**
www.hotelparkbeograd.rs

Председник
Организационог одбора

Др Милош Лукић
Србија

Председник
Међународног научног комитета

Проф. др Martin Wahner
Немачка