

12th
INTERNATIONAL
SYMPOSIUM

MODERN
TRENDS
IN LIVESTOCK
PRODUCTION



P R O C E E D I N G S

9 -11 October 2019, Belgrade, Serbia

Institute for Animal Husbandry

Belgrade - Zemun, SERBIA

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Language editor

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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

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Institute for Animal Husbandry
Autoput 16, P. Box. 23,
11080, Belgrade-Zemun, Serbia
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Address:

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Autoput 16, P. Box 23,
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Tel:

+381 11 2691 611

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INVITED PAPERS

AGRO BIODIVERSITY AND LIVESTOCK FARMING: AUTOCHTHONOUS SPECIES AND BREEDS IN SERBIA

Čedomir Radović¹, Marija Gogić¹, Dragan Radojković², Vladimir Živković¹, Nenad Parunović³, Aleksandar Stanojković¹, Radomir Savić²

¹ Institute for Animal Husbandry, Autoput 16, 11080, Belgrade-Zemun, Republic of Serbia

² University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080, Belgrade-Zemun, Republic of Serbia

³ Institute of Meat Hygiene and Technology, Kačanskog 13, 11000, Belgrade, Republic of Serbia

Corresponding author: Čedomir Radović, cedomirradovic.izs@gmail.com

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Abstract: Agro biodiversity or genetic resources for agriculture and food is one of the most significant component of total biodiversity, and it encompasses various animals, plants and micro-organisms that are necessary to maintain the key functions of agro-ecosystems. According to the United Nations, the world population has reached as much as 7.3 billion to date, with a forecast of 9.7 billion in 2050 and 11 billion by the end of the century. Well-adapted and well-developed livestock farming is an essential element of the agricultural production system, especially important in difficult conditions when crop production in the agro-ecosystem cannot sustain and increase its productivity and adapt to changing circumstances, and is crucial for the food security of the population. Preservation and improvement of livestock farming, animal genetic diversity, preservation of autochthonous and development of new breeds, preservation of genetically diverse populations provide society with a greater range of options to meet the future challenges in the development of agriculture. This paper gives review of the condition of farm animals and autochthonous breeds: Podolian bovine, Busha, domestic buffalo, domestic-mountain horse, Nonius, Balkan donkey, Mangalitsa, Moravka, and Resavka pig, Pirot sheep, Bardoka, Krivovir sheep, Karakachan sheep, Lipska sheep, Vlačka-Vitoroga sheep, Čokan Tsigai goat, Balkan goat, Svrlijig hen, Banat Naked Neck hen and Sombor Kaporka hen. Genetic resources in animal husbandry represent all species, breeds and strains of farm animals and they have economic, scientific and cultural significance for our country.

Key words: biodiversity, autochthonous species, autochthonous breeds, genetic resources

Introduction

Agricultural biodiversity is the product of thousands of years of activity in which people have tried to meet their needs within a wide range of social, climatic and environmental conditions. According to the United Nations Population Division (2019), the world population has reached 7.3 billion today. The world's population is expected to increase by 2 billion people in the next 30 years, from 7.7 billion currently to 9.7 billion in 2050, according to a new United Nations report launcher today. According to projections, as many as 11 billion people will live on Earth by the end of the century. The UN indicates that by 2050, half of the world's population will live in nine countries: India, Pakistan, Nigeria, Congo, Ethiopia, Tanzania, the United States, Indonesia and Uganda, cited by size for their contribution to overall population growth. It is also estimated that India will exceed China in terms of population over the forecast period.

Europe, on the other hand, is facing the phenomenon of global aging and is estimated to have more than 34 percent of the population over 60 by 2050. The UN also predicts a population decline in eastern European countries, including Serbia, as well as neighboring countries Croatia, B&H, Hungary, Bulgaria and Romania. The UN population forecast is generally projected to decline in 48 countries. The estimated population in the Republic of Serbia in 2018 is 6.982.604, which is by 5.5% less than in the previous year.

Well-adapted and well-developed livestock farming is an essential element of the agricultural production system, especially important in difficult conditions where crop farming in the agro-ecosystem cannot sustain and increase its productivity and adapt to changing circumstances, and is crucial for the food security of the population. Preservation and improvement of livestock farming, animal genetic diversity, preservation of autochthonous and development of new breeds, preservation of genetically diverse populations provide society with a greater range of options to meet the future challenges in the development of agriculture. Genetic selection may lead to loss of homeostatic balance of animals, resulting in the occurrence of pathologies and consequently in impaired animal welfare (*Petrović et al. 2017*). Responsible management of agricultural biodiversity worldwide is becoming a growing challenge for the international community. Especially in the livestock sector, as it is undergoing dramatic changes, intensifying production by trying to meet the increasing demands for increased production of meat, milk and eggs.

Significance of agro biodiversity

Agrobiodiversity or genetic resources for food and agriculture is one of the most significant component of total biodiversity, and it encompasses various

animals, plants and micro-organisms that are necessary to maintain the key functions of agro-ecosystems.

Agrobiodiversity is the result of the interaction of the environment, genetic resources, management systems and how it is practiced.

Numerous plant varieties, also breeds and strains of animals have emerged as a result of multi-millennial natural selection on the one hand, and planned selection and executed by men, on the other.

The growing of cultivated plants and the domestication of animals, together with the invention of fire, represent the most remarkable achievements of humanity and form the three main pillars upon which human civilization and its development rest.

Numerous studies and experiences indicate that the richness of agrobiodiversity contributes to:

- increase of production,
- secure food supply and economic profit,
- reducing agricultural pressure on poorer - barren areas, forests and endangered species,
- development of stable and sustainable agricultural production,
- sustainable intensification of agricultural production,
- increasing the efficiency of the use of available resources and the environment,
- improving the quality of human nutrition,
- reducing the risk of pests and diseases,
- preserving the soil and increasing its natural fertility and
- preserving ecosystem structure and species diversity.

During the twentieth century, many structural changes occurred at the level of global agrobiodiversity. About 75% of plant genetic diversity has been lost mainly because farmers replaced many autochthonous varieties with genetically more uniform and high-yielding varieties. Also, about 30% of the breeds in the livestock sector are at risk of complete disappearance, and an average of six breeds are lost every month. Climate change and the occurrence of new animal diseases require the preservation and conservation of plant and animal resources due to their adaptive capacity. For hundreds of millions of poor rural households, livestock and crop plant farming is a key resource for life. According to FAO records, almost 75% of present food production is obtained from only 12 species of plants and 5 species of animals. To meet food needs, the human population uses only 150 to 200 of the total of 250,000 to 300,000 edible plant species, of which three (rice, corn and wheat) account for 60% of the calories and proteins derived from plant foods. On the other hand, farmed animals provide 30% of the human population's food

needs. According to FAO estimates, the demands and needs for animal products in the world will increase by 20-30% in the next 20-30 years. Autochthonous breeding also encourages the revitalization of rural areas, providing the autochthonous population with additional sources of income, suitable for the use and maintenance of grazing land and preventing the devastation and succession of natural habitats. Recently, interest in native autochthonous breeds has increased not only for the purpose of gene preservation but also the production of meat products manufactured in the traditional way (*Petrović et al. 2010*).

The state of animal genetic resources in the world

According to the FAOSTAT (2006) Global Database for Animals Genetic Resources, there are 7616 breeds of various species of domestic animals (10 species of mammals and eight species of birds - poultry), of which 6536 are autochthonous (autochthonous) races and 1080 are widespread, regionally or globally (these are usually high-yielding breeds). According to the same report, 20% of the total number of breeds listed are at risk of complete disappearance (effective population size less than 100 individuals). In the period from 1999 to 2006 alone, 690 breeds disappeared, of which 9 were highly productive and all others were autochthonous breeds. By species, 209 breeds of cattle, 19 breeds of goats, 87 breeds of horses, 140 breeds of pigs, 180 breeds of sheep and 6 breeds of donkeys have disappeared.

Given the above data, there is a growing emphasis on the general - global need to study and conserve all species, breeds and strains of livestock, especially autochthonous ones. Primarily because of their importance, as the carrier of specific genes necessary to create new breeds and genetic combinations for immediate exploitation, as well as to preserve the gene pool needed for future generations.

In order to preserve autochthonous breeds and strains, extensive efforts are being made and significant financial resources are being invested worldwide through various international institutions, notably the United Nations Food and Agriculture Organization (FAO), as well as numerous regional and national organizations.

To this end, FAO (1992) made recommendations for the preparation of the Global Animal Genetic Resources Management Program, on the basis of which a document entitled: Global Strategy for the Management of Farm Animal Genetic Resources was prepared and adopted in 1993.

In accordance with the recommendations of this strategy, a Global Domestic Animal Diversity Information System (DAD-IS) has been established and national and regional focal points designated.

In 2001, the FAO launched an initiative to produce Member States' National Reports on the state of animal genetic resources, their contribution to the development of agriculture and rural areas and the national capacity to manage and conserve genetic resources (FAO, 2001). Based on 169 national reports collected, out of a total of 188 FAO member countries, the first global report was prepared: The state of animal genetic resources for food and agriculture in the world, as well as the report: A priority strategy for activities for the sustainable use, development and conservation of animal genetic resources for food and agriculture.

First Technical Conference on Animal Genetic Resources and Declaration adopted: Global Action Plan for Animal Genetic Resources, defining global priorities for further conservation of genetic resources in livestock (FAO, 2007).

Livestock situation in the Republic of Serbia

Table 1 shows the number of cattle, pigs, sheep and poultry from 1999 to 2018. We see that in the period from 1999 to 2018, the number of cattle decreased by 31.6%, pigs by 35.2% and poultry by 30.3%, while the only increase recorded was in the number of sheep by 7.1%.

Table 1. Number of livestock and poultry in Serbia since 1999 (in 000)

Year	Cattle	Index	Pigs	Index	Sheep	Index	Poultry	Index
1999	1283	100.0	4293	100.0	1598	100.0	23278	100.0
2000	1246	97.1	4066	94.7	1611	100.8	20360	87.5
2002	1128	87.9	3587	83.6	1448	90.6	18804	80.8
2004	1102	85.9	3439	80.1	1586	99.2	16280	69.9
2006	1106	86.2	3999	93.2	1556	97.4	16595	71.3
2008	1057	82.4	3594	83.7	1605	100.4	17188	73.8
2009	1002	78.1	3631	84.6	1504	94.1	22821	98.0
2010	938	73.1	3489	81.3	1475	92.3	20156	86.6
2011	937	73.0	3287	76.6	1460	91.4	19103	82.1
2012	921	71.8	3139	73.1	1635	102.3	18234	78.3
2013	913	71.2	3144	73.2	1616	101.1	17860	76.7
2014	920	71.7	3236	75.4	1748	109.4	17167	73.7
2015	916	71.4	3284	76.5	1789	111.9	17450	75.0
2016	893	69.6	3021	70.4	1665	104.2	16242	69.8
2017	899	70.0	2911	67.8	1704	106.6	16338	70.2
2018	878	68.4	2782	64.8	1712	107.1	16232	69.7

Source: Statistical Office of the Republic of Serbia (2019)

According to the data presented in the report of the Statistical Office of the Republic of Serbia (2019), as of December 1st, 2018, as compared to the previous situation, the trend of decrease in the number of cattle, pigs and livestock has

continued, in case of cattle by 2.3%, pigs by 4.1% and poultry by 0.7%, while the total number of sheep increased by 0.4%. Cattle are mostly reared in the Region of Šumadija and Western Serbia (46.7% compared to the total number of cattle in the territory of the Republic of Serbia), and pigs in the Region of Vojvodina (44.3%).

In Table 2 we can see that in 2018 milk production decreased by only 4.4% compared to 2008 in spite of the decrease in the total number of cattle (Table 1) by 16.9%. Sheep milk production is up by 5.9% in 2018 compared to 2008, as is the number of sheep, which is by 6.6% higher than the number of sheep in 2008. Of particular note is the increase in honey production in 2018 by 274.4% compared to 2008.

Table 2. Production of milk, eggs, honey and wool

Year/Product	Cow milk total, mil. Lit.	Sheep milk total, mil. Lit.	Goat milk total, mil. Lit.	Eggs, mil. pieces	Honey, t	Wool, t
2008	1561	14	36	1726	4164	2596
2009	1505	10	28	1711	7354	2403
2010	1485	10	27	1705	7281	2461
2011	1462	11	29	1760	6963	2385
2012	1465	12	33	1794	6983	2662
2013	1451	18	34	1755	8554	2720
2014	1492	20	38	1892	4383	2687
2015	1501	19	44	2061	12263	2769
2016	1504	17	37	1853	5761	2848
2017	1506	14	33	1759	7014	2831
2018	1493	18	34	1796	11427	2844

Source: Statistical Office of the Republic of Serbia (2019)

Table 3 shows the meat production in the Republic of Serbia for ten years. Beef production declined by 23.23% over the ten year period, while the population decreased by 16.9% over the same period. Increase in pork production compared to 2008 is by 13.9% although the number of pigs is lower by 22.6% compared to 2008, which can be interpreted by the change of breed composition, more fertile and meaty genotypes, especially on smaller farms. Sheep and poultry meat also has an increasing trend compared to 2008.

Table 3. Meat production (in 000 t)

Year/Product	Beef	Pork	Mutton	Poultry
2008	99	266	23	76
2009	100	252	24	80
2010	96	269	23	84
2011	81	271	24	103
2012	82	252	22	94
2013	70	249	30	92
2014	73	258	27	94
2015	77	278	30	86
2016	77	301	34	88
2017	71	307	30	95
2018	76	303	32	106

Source: Statistical Office of the Republic of Serbia (2019)

Table 4 contains data on population size and number of breeders in the Republic of Serbia for 2018. The largest number of breeds within the species is found in sheep breeding, and looking at the individual breeds, the largest population is Mangalitsa with 2105 heads.

Table 4. Autochthonous breeds of domestic animals, population size and number of breeders

Species	Breed	Population size (number of breeding animals under control)	Number of breeders
Bovine	Busha	1274	23
	Podol cattle	304	89
Buffalo	Domestic buffalo	1031	322
Horse	Domestic mountain horse	1039	334
	<i>Nonius</i>	91	23
Donkey	Balkan donkey	541	59
Pig	Mangalitsa	2105	100
	Moravka	402	35
	Resavka	44	6
Sheep	Bardoka	198	7
	Krivovir sheep	1112	38
	Pirot sheep	194	11
	Karakačan sheep	213	7
	Lipska sheep	1302	39
	Vlaško-Vitoroga sheep	838	16
	Čokan Tsigai	1236	19
Goat	Balkan goat	781	25
Poultry	Svrljig hen	82	1
	Sombor Kaporka hen	273	4
	Banat Naked Neck hen	522	5

Source: Ministry of Agriculture, Forestry and Water Management

The population trend of autochthonous breeds of domestic animals for the period 2000–2018 is shown in Table 5.

Table 5. Population trend of autochthonous breeds of domestic animals in the Republic of Serbia for the period 2000–2018

<i>Species</i>	<i>Breed</i>	<i>2000</i>	<i>2005</i>	<i>2010</i>	<i>2015</i>	<i>2018</i>
Bovine	Busha	-	32	750	669	1274
	Podol cattle	110	134	350	240	304
Buffalo	Domestic buffalo	/	93	800	423	1031
Horse	Domestic mountain horse	9	19	80	110	1039
	<i>Nonius</i>	17	34	90	74	91
Donkey	Balkan donkey	/	10	250	281	541
Pig	Mangalitsa	124	254	400	780	2105
	Moravka	/	32	100	103	402
	Resavka	/	3	40	16	44
Sheep	Bardoka	/	89	300	687	1302
	Krivovir sheep	/	258	350	532	1112
	Pirot sheep	/	30	60	81	198
	Karakačan sheep	/	74	400	468	838
	Lipska sheep	/	/	60	101	194
	Vlaško-Vitoroga sheep	/	/	130	165	213
	Čokan Tsigai	100	376	450	650	1236
Goat	Balkan goat	242	157	250	521	781
Poultry	Svrljig hen	/	113	200	250	82
	Sombor Kaporka hen	100	352	250	260	273
	Banat Naked Neck hen	50	222	900	560	522

Source: Ministry of Agriculture, Forestry and Water Management

Organisations for breeding, monitoring, and conservation

Breeding is regulated by the Law on Livestock (2016). Institute of Animal Husbandry, University of Belgrade Faculty of Agriculture and the University of Novi Sad, Faculty of Agriculture are the authorized Head Breeding Organizations for the selection and recording of breeding livestock in Serbia. The conservation of LAGs in the Republic of Serbia is defined by a series of regulations and by-laws: the Agriculture and Rural Development Strategy 2014-2024, the National Rural Development Program 2018-2020, the Law on Ratification of the Convention on Biological Diversity, the Livestock Act, the Law on Agriculture and Rural Development, the Law on incentives in agriculture and rural development. Of the by-laws of importance are: the Rulebook on the List of Genetic Reserves of Domestic Animals, the Method of Preserving the Genetic Reserves of Domestic Animals, and the List of Indigenous Breeds of Domestic Animals and Endangered

Indigenous Breeds and the method of keeping a register of breeders of indigenous breeds of domestic animals, the Rulebook on incentives for the conservation of animal genetic resources, and the Ordinance on incentives for the conservation of animal genetic resources in the gene bank. *Ex-Situ* preservation is regulated by the Rulebook on incentives for the conservation of animal genetic resources in the gene bank (2017).

Conservation methods for animal genetic resources

The conservation of autochthonous and adapted species or animal genetic resources (AnGR) is a major problem, but even more worrying is that some AnGRs were lost before their characterization and their potential has been explored. No nuclei with adequate numbers of animals were formed (*in-situ* conservation) and there was no cryopreservation of the embryos and *ex-situ* conservation for the lost autochthonous breeds. The population trend of autochthonous breeds of domestic animals by nucleus formation with an appropriate number of animals (*in-situ* conservation) has stabilized and is on the rise. We have also started *ex situ* conservation and there is now a legal framework for that. Conservation strategies that involve both *in situ* measures (supporting the maintenance of livestock populations in their usual production environments) and cryo-conservation (storage of frozen genetic material) are widely discussed as the optimal means of protecting threatened breeds from extinction. In vitro gene banks have been established by 64 countries and a further 41 countries are planning to do so (FAO, 2015). The very concept of animal genetic resources denotes all species, breeds and strains of scientific, cultural and economic importance to a single country. Particular attention should be paid to the conservation of native breeds of domestic animals, because of the risk of their extinction and extinction. These breeds represent an important source of genetic potential for future livestock work.

Conclusion

Given that, according to projections, as many as 11 billion people are expected to live on Earth by the end of the century, livestock production needs to be promoted, populations increased and more productive breeds created while preserving the quality of the end product. With regard to autochthonous breeds, *in situ* and *ex situ* conservation should be increased so that they are not endangered. The last decade has been marked by a much larger number of scientific and professional publications on autochthonous species and breeds of domestic animals. It is necessary to form a national gene bank *in situ* and *ex-situ* because

autochthonous breeds have specific genes and are more resistant to certain diseases, more stress tolerant, or not as sensitive as allochthonous commercial breeds and better adapt to climate change. In this regard, natural disasters and the coming climate change can be a great challenge to preserve agro biodiversity.

In addition to the National Gene Bank, further alignment of national legislation with the regulations of the European Union and other international organizations is required.

When it comes to breeding allochthonous-commercial species, the fact that the population of cattle, pigs and poultry has fallen by more than 30% in the last 18 years is worrying. In contrast, the fact that the number of breeders and heads of autochthonous breeds has increased in recent years is encouraging. The reason for the increase is primarily due to incentive measures for cultivation by the Ministry of Agriculture, Forestry and Water Management, marketing and market.

The richness and diversity of the living world – biodiversity, is a distinctive feature of the Republic of Serbia. Extremely rich genetic resources of plants and animals, as well as a large number of varieties and breeds, and especially autochthonous plant and animal populations obtained through centuries-old, mostly natural selection, are very significant resources of Serbia, that is, the biological basis for agriculture and food production.

Agrobiodiverzitet i stočarstvo: lokalne vrste i rase u Republici Srbiji

Čedomir Radović, Marija Gogić, Dragan Radojković, Vladimir Živković, Nenad Parunović, Aleksandar Stanojković, Radomir Savić

Rezime

Agrobiodiverzitet ili genetički resursi za poljoprivredu i hranu predstavljaju jednu od najznačajnijih komponenti ukupnog biodiverziteta, a obuhvata razne životinje, biljke i mikroorganizme koji su neophodni za održavanje ključnih funkcija agro-ekosistema. Prema podacima Ujedinjenih nacija svetska populacija je do danas dostigla čak 7,3 milijardi predviđanje je da će 2050. godine biti 9.7 milijardi a do kraja veka 11 milijardi. Dobro prilagođeno i razvijeno stočarstvo je bitan element sistema poljoprivredne proizvodnje, posebno je važno u teškim uslovima kada ratarstvo u agro-ekosistemu ne može da održi i poveća svoju produktivnost i da se prilagodi promenljivim okolnostima, i od ključnog je značaja za prehrambenu sigurnost stanovništva. Očuvanje i unapređenje stočarstva, životinjskih genetskih

različitosti, očuvanje autohtonih i razvoj novih rasa, očuvanje genetski različitih populacija pružaju društvu veći opseg opcija u susret budućim izazovima i razvoju poljoprivrede. U ovom radu je prikazano brojno stanje domaćih životinja i stanje autohtonih rasa: podolskog govečeta, buše, domaćeg bivola, domaćeg-brdskog konja, noniusa, balkanskog magarca, mangulice, moravke, resavke, pirotke ovce, bardoke, krivovirske ovce, karakačanske ovce, lipske ovce, vlaško-vitoroge ovce, čokanske cigaje, balkanske koze, svrljiške kokoši, banatskog gološijana i somborske kaporke. Genetički resursi u stočarstvu predstavljaju sve vrste, rase i sojeve domaćih životinja i one imaju ekonomski, naučni i kulturološki značaj za našu zemlju.

Ključne reči: biodiverzitet, lokalne vrste, lokalne rase, genetički resursi

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THE FUTURE OF AGRICULTURE PRODUCTION – COULD THE FORECASTED EVENTS BE ALTERED?

Vesna Gantner, Irena Jug

Faculty of Agrobiotechnology Osijek, University of Josip Juraj Strossmayer in Osijek, Osijek, Croatia

Corresponding author: Vesna Gantner, vgantner@fazos.hr

Invited paper

Abstract: Currently we are living in the world characterised by a rapid changes of climate and increasing food demands for an ever-growing human population. The population growth, combined with rising incomes and urbanization, will impose great challenges to systems of food production and agriculture. On the other hand, the natural resources necessary to enable global food and agricultural production will not grow. Therefore, necessary production growth must be accommodated within the growing insufficiency of natural resources (land, water and nutrients). Furthermore, in order to minimize the impact of global food and agricultural production on environment, waste and greenhouse gasses (GHG) emissions must be reduced. The global agriculture, and especially animal production sector significantly contributes to an anthropogenic GHG emissions, but at the other hand, it can also deliver a significant share of the necessary mitigation effort. If the goal is sustainable food production, it is necessary to design and implement cost-effective and just mitigation strategies. All the sector stakeholders, private and public sector, civil society, research and academia, and international organizations, have to be involved in problem solving. Mitigation actions implies well planned, effective and fair strategies and policies based on global approach. In order to enable sustainable agriculture and rural development European Commission proposed post-2020 Common Agricultural Policy. The questions are: shouldn't we just "accept" climate change and a world with 9.6 billion people by year 2050? Could the forecasted events be altered?

Key words: agriculture, animal production, common agricultural policy, climate change

Introduction

Currently we are living in the world characterised by a rapid changes of climate and increasing food demands for an ever-growing human population. The forecasts indicate that the world population will increase from current 7.2 billion to 9.6 billion till year 2050 (Figure 1; US Census Bureau, 2016).

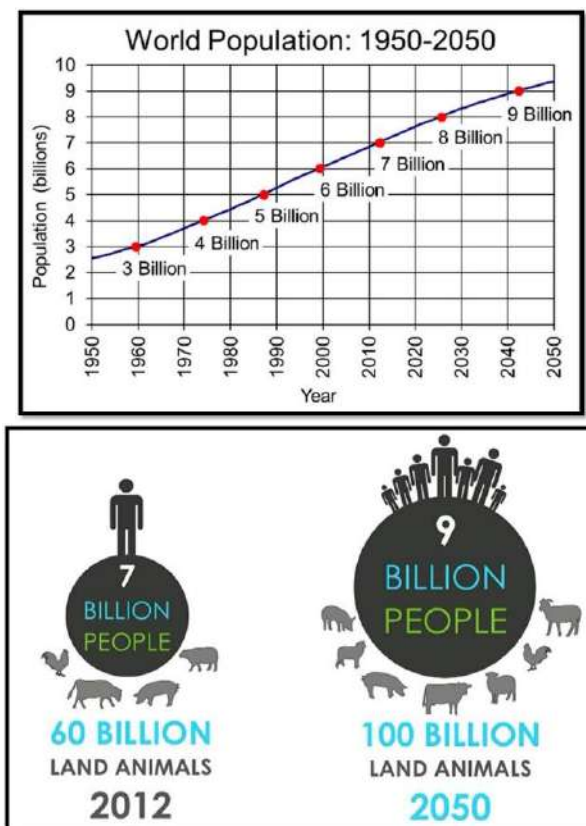


Figure 1 Population growth
(US Census Bureau, 2016)

Picture 1 Expected increase of land animals
(FAO, 2011)

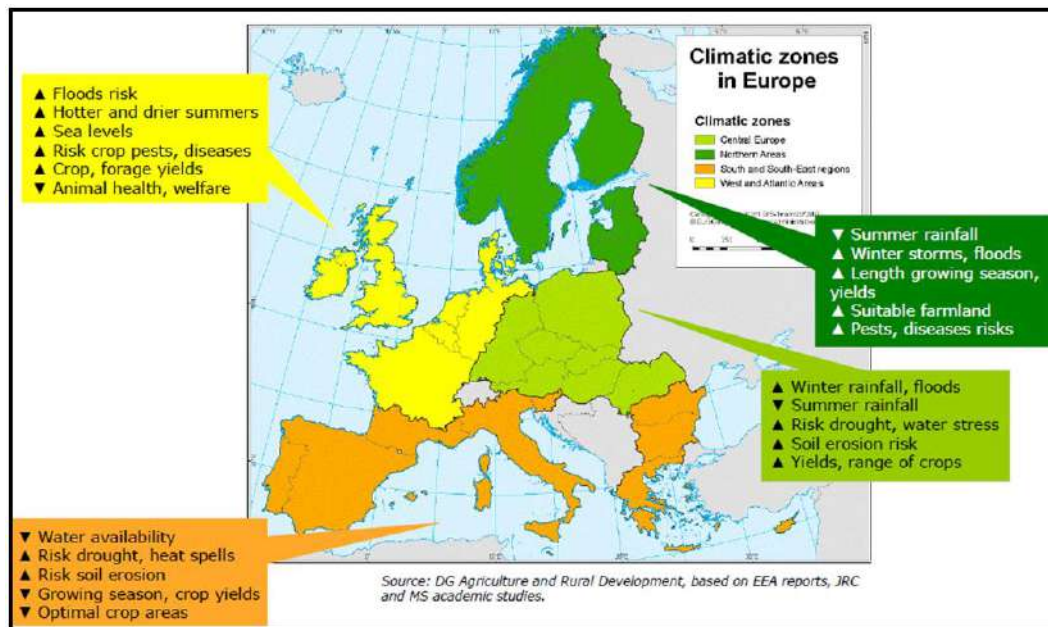
Accordingly, to *FAO (2011)* population growth, combined with rising incomes and urbanization, will impose great challenges to systems of food production and agriculture. The increase in animal-source foods will be especially expressed. The forecasts indicate that the demand for meat and milk in year 2050, regarding the levels in 2010, will increase by 73 and 58% (*FAO, 2011*). Predicted increase of human population implies an increase in the population of land animals from current 60 billion to 100 billion till year 2050 (Picture 1).

The natural resources necessary to enable global food and agricultural production will not grow. Therefore, necessary production growth must be accommodated within the growing insufficiency of natural resources (land, water and nutrients). Furthermore, in order to minimize the impact of global food and agricultural production on environment, waste and greenhouse gasses (GHG)

emissions must be reduced. *FAO (2013)* experts stated that climate change is transforming the planet's ecosystems and threatening the well-being of current and future generations, therefore, with purpose to *hold the increase in global temperature below 2°C* and to avoid *dangerous* climate change, global emissions need to be significantly decreased. The latest findings (*IPCC, 2018*) emphasised that a number of climate change impacts could be avoided in a case of limiting the global warming to 1.5°C compared to 2°C, for instance lower global sea level rise. Limiting global warming would also give people and ecosystems more room to adapt and remain below relevant risk thresholds. Limiting global warming to 1.5°C would require rapid, far reaching and unprecedented changes in all aspects of society.

European food production – future challenges

Agriculture worldwide in the future period will be faced with a number of major challenges, for instance: rapid population growth, an increasing demand for energy, resource shortages, accelerated urbanisation, dietary changes, ageing populations in rural areas in developed countries, increased competition on world markets, lack of access to credit developing countries, and the most pronounced one, climate change (*EPRS, 2016*). How will agriculture in the European Union be affected by climate change? Accordingly, to *EC, DG AGRI (2016)* the changing in rainfall will be a serious problem in many European regions. Also, rising temperatures, variability and seasonality as well as extreme events, heatwaves, droughts, storms and floods across the EU are just some of the expected changes (Picture 2).



Picture 2 Impact of climate change on European Union (EC, DG AGRI, 2016)

Furthermore, to the *IPCC (2007)* changes in climate will significantly affect animal production worldwide. In support to stated, *Battisti and Naylor (2009)* forecasted that by year 2050, most of the world will be exposed to median temperatures in the summer that will be higher than the highest recorded temperatures. Furthermore, *Reiczigel et al. (2009)* in Hungary, as well as *Dunn et al. (2014)* in UK indicated an increase of heat stress days per year.

Animal production in changing environment

Today's milk production is characterized by high cattle productivity requirements and increasingly pronounced unfavourable (micro)climate conditions. *Gauly et al. (2013)* emphasized that the heat stress of high productive dairy cows will cause growing concern in dairy production in Europe, while *Segnalini et al. (2013)* pointed out the necessity of adequate adaptation strategies in order to decrease negative effects of climate change on domestic animals. *Hansen (2013)* stated that the high production makes cows more susceptible to heat stress meaning that heat stress will become, and already is, a problem in intensive dairy breeding systems regardless the climate changes. The intensive genetic selection for high milk production resulted in changes in the thermoregulation physiology of dairy cattle (*Kadzere et al., 2002*). Larger frames and larger gastrointestinal tracts in high

production animals enable them to digest more feed, but also creates more metabolic heat and reduces the animal's ability to regulate temperature heat stress environment meaning that increased milk yield, feed intake and metabolic heat the thermoneutrality to lower temperatures. Accordingly, to many studies, heat stress conditions in dairy cattle reduce dry matter intake, milk production as well as reproductive performances. Furthermore, heat stress alters the milk composition, somatic cell counts (SCC) and prevalence of mastitis (*Gantner et al. 2011, 2017; St-Pierre et al. 2003; Smith et al. 2013*). Finally, heat stress induces considerable profit loss (*St-Pierre et al. 2003*). The most common measure of heat stress in dairy cattle is temperature-humidity index (THI) that includes ambient temperature and relative humidity (*Kibler 1964*). The THI threshold value at which heat stress affects dairy cattle that is milk production and feed intake vary, depending on study, from 68 to 72 (Picture 3).

Temperature Humidity Index (THI)									
	Relative Humidity %								
C	20	30	40	50	60	70	80	90	100
22	66	66	67	68	69	69	70	71	72
24	68	69	70	70	71	72	73	74	75
26	70	71	72	73	74	75	77	78	79
28	72	73	74	76	77	78	80	81	82
30	74	75	77	78	80	81	83	84	86
32	76	77	79	81	83	84	86	88	90
34	78	80	82	84	85	87	89	91	93
36	80	82	84	86	88	90	93	95	97
38	82	84	86	89	91	93	96	98	100
40	84	86	89	91	94	96	99	101	104

No heat stress

Moderate heat stress

Severe heat stress

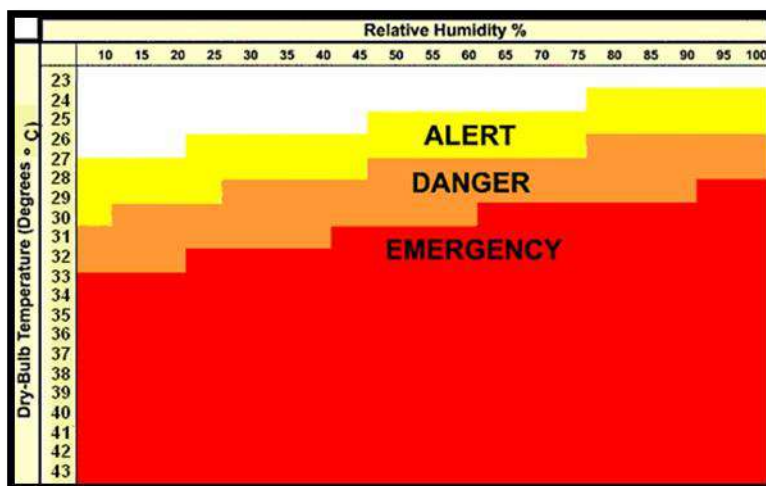
Dead cows

Picture 3 Heat stress status of dairy cows depending of THI value of environment (Farm health online, 2019)

There are many methods for reduction of heat stress, short-term, such as shading, cooling and nutrition, and long-term that is a selection for heat stress resistance.

Heat stress also induce set of serious problems in pig breeding. Since pigs do not sweat and have relatively small lungs they are prone to heat stress. In a case of heat stress environment pigs tends to increase respiration rate and loss the appetite (*DPIRD, 2019*). If heat stress continues, pigs start to drink excessive amounts of

water. Furthermore, in heat stress environment, fatteners tend to decrease the average daily gain. The amount of decrease depends of animal's weight as well as ambient temperature (75kg pigs tend to decrease the ADG at temperatures above 23°C, while in 25kg pigs ADG starts to decrease when exposed to temperatures above 27°C). Besides ambient temperature, air humidity also contributes to heat stress and pigs generally develop heat stress at much lower temperatures when the humidity is high (Picture 4).



Picture 4 Heat stress status of fatteners depending of THI value of environment (DPIRD, 2019)

Accordingly, to *DPIRD (2019)* recommended management tools to reduce heat stress are as follows: increase ventilation and airflow and regularly check cooling system is in good working order (for example spray cooling), reduce stocking density if possible, maintain drinking water temperature as low as possible (around 10°C is ideal but difficult to achieve), avoid feeding between 10.00am-4.00pm (the hottest period of the day), supplement electrolytes and antioxidants through the water supply, increase dietary energy density, minimise excess non-essential amino acids and fibre (minimising intestinal fermentation and therefore heat production), and increase availability of antioxidants through the diet such as vitamin E and betaine.

The global agriculture, and especially animal production sector significantly contributes to an anthropogenic GHG emissions, but at the other hand, it can also deliver a significant share of the necessary mitigation effort. The need to reduce the sector's emissions and its environmental impact has become ever more pressing in

view of its continuing expansion to ensure food security and feed a growing, richer and more urbanized world population. Application of different mitigation techniques in livestock production sector could result in large environmental benefits. The mitigation potential varies in interval from 14 to 41% depending on the selected species, production system and world's region (*FAO, 2013a,b*). If the goal is sustainable food production, it is necessary to design and implement cost-effective and just mitigation strategies. All the sector stakeholders, private and public sector, civil society, research and academia, and international organizations, have to be involved in problem solving. Mitigation actions imply well planned, effective and fair strategies and policies based on a global approach.

The post-2020 Common Agricultural Policy: environmental benefits and simplification

What the future CAP will bring to the table for the environment and climate - key elements:

1. A needs-based, targeted approach to addressing environmental and climate objectives through the whole CAP in coherence with other EU policies
 - Three out of nine of the CAP's objectives to cover the environment and climate
 - Assessment of needs, targeting and performance monitoring to cover both CAP "pillars" (direct payments to farmers and support for rural development) in a single plan per Member State, for greater coherence
 - New link to other EU legislation on the environment and climate
2. An improved system of conditions ("conditionality") to be met by farmers receiving area- and animal-based CAP payments
 - Member States to plan implementation to match overall CAP objectives and national assessment of needs – with approval by Commission
 - A small number of new standards, strengthened existing standards
3. A complementary set of (voluntary) tools to be offered to farmers to help achieve the CAP environmental and climate objectives
 - A new stream of funding for the environment and climate ("eco-schemes") from the CAP's direct payments budget, mandatory for Member States (but design up to them) and voluntary for farmers
 - Continued support from the CAP's rural development budget for environment- and climate-relevant management practices, investments, knowledge-building, innovation and co-operation. Ongoing wide range of support possibilities, with at least 30% of the CAP's rural development

budget to focus on activities of most direct value for the environment and climate.

Instead of a conclusion

Sustainable agriculture in a light of climate change and nine billion mouths to feed by year 2050? We shouldn't just "accept" climate change, by intensification of agricultural production and application of adequate mitigation methods emissions from agricultural production could be reduced up to 40%. Furthermore, we shouldn't just "accept" a world with 9 billion people by year 2050. Currently, the world population is growing, but fertility rates, in many states, significantly decreased over the last few decades. In order to prevent excessive growth of human population, education and reproductive health services should be significantly emphasized. Finally, research and innovation has made the European agricultural sector competitive, balanced and efficient as it is today. Therefore, significant investment could enable research breakthroughs that are the key factor for securing the sustainability and competitiveness of the food production systems in the future.

Budućnost poljoprivredne proizvodnje - da li bi se prognozirani događaji mogli izmeniti?

Vesna Gantner, Irena Jug

Rezime

Trenutno živimo u svetu koji karakterišu brze promene klime i sve veće potrebe za hranom za sve veću ljudsku populaciju. Rast stanovništva u kombinaciji sa rastućim prihodima i urbanizacijom nameće velike izazove sistemima proizvodnje hrane i poljoprivrede. S druge strane, prirodni resursi potrebni za omogućavanje globalne proizvodnje hrane i poljoprivrede neće rasti. Stoga se neophodni rast proizvodnje mora uskladiti sa rastućom insuficijencijom prirodnih resursa (zemlje, vode i hranljivih sastojaka). Nadalje, da bi se minimizirao uticaj globalne prehranbene i poljoprivredne proizvodnje na životnu sredinu, otpad i gasovi staklene bašte (greenhouse gasses - GHG) moraju biti smanjeni. Globalna poljoprivreda, a posebno sektor stočarstva, značajno doprinosi antropogenim emisijama gasova staklene bašte, ali s druge strane, takođe može pružiti značajan deo neophodnih napora za ublažavanje. Ako je cilj održiva proizvodnja hrane, potrebno je osmisлити i implementirati ekonomične i pravedne strategije ublažavanja. U rešavanje problema moraju biti uključeni svi akteri iz sektora, privatni i javni sektor, civilno društvo, istraživači/nauka i akademije, kao i

međunarodne organizacije. Akcije ublažavanja podrazumevaju dobro isplanirane, efektivne i fer strategije i politike zasnovane na globalnom pristupu. Kako bi se omogućila održiva poljoprivreda i ruralni razvoj Evropska komisija je predložila Zajedničku poljoprivrednu politiku nakon 2020. godine. Pitanja su: zar ne bismo samo trebali „prihvatiti“ klimatske promene i svet sa 9,6 milijardi ljudi do 2050. godine? Mogu li se izmeniti prognozirani događaji?

Ključne reči: poljoprivreda, stočarska proizvodnja, zajednička poljoprivredna politika, klimatske promene

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GENERAL PRINCIPLES AND GOOD ANIMAL WELFARE PRACTICES ON DAIRY CATTLE FARMS

Slavča Hristov¹, Dušica Ostojić Andrić², Branislav Stanković¹

¹University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Zemun, Serbia

²Institute for Animal Husbandry, Belgrade – Zemun, 11080 Zemun, Serbia

Corresponding author: Slavča Hristov, hristovs@agrif.bg.ac.rs

Invited paper

Abstract: Field experiences and scientific observations point out the need to define a guide which would describe widely applicable general principles and good practices for ensuring the welfare of dairy cattle. The key action areas in the guide should be stockmanship, feed and water, living environment, husbandry practices and health management. There is a set of principles in each of these key action areas that should be used to further define the good practice of ensuring dairy cattle welfare in farms. The most important issue for the implementation of good welfare practices for dairy cows is stockmanship. Appropriate nutrition is a basic requirement and it is considered to have a great role to ensure good dairy cattle welfare. Environment, space, equipment, microclimatic and hygienic conditions significantly influence the welfare of dairy cattle. Dairy cattle should be treated with care and in a consistent manner, taking into account their natural behaviour all the time, and in any case minimizing the risk of injury and distress. For the welfare of dairy cattle of particular importance are many health conditions as: lameness, mastitis, injuries, acidosis, milk fever and other metabolic diseases, diarrhoea and anaemia of calves, respiratory diseases, heat stress, arthritis and many contagious diseases. It is necessary to introduce systematic training of stockpersons in our country with special attention to stockmanship and responsibility, feed and water, living environment, husbandry practices and health management in order to achieve a stable system of ensuring good dairy cattle welfare.

Key words: dairy cattle welfare, general principles, good welfare practice

Introduction

Nowadays dairy cattle farms of different capacities are present in Serbia. The herd size ranges from several dairy cows and related categories in individual farms to several thousand in modern industrial farms. There are different systems

for rearing, accommodation and feeding of these animals. Also, in these production systems, there are different combinations from rearing the animals entirely in the barns to the simultaneous use of barns and pastures in different forms (*Hristov and Stanković, 2016*).

Today, ensuring the welfare of dairy cattle, in essence, the quality of life they have at any given moment is one of the key issues in milk production (*Hristov et al., 2012a; von Keyserlingk et al., 2014*). Ensuring the welfare of animals is largely dependent on farm size (*Robbins et al., 2016*), holding and accommodation system (*Ostojić-Andrić et al., 2011*), nutrition, use of pastures, husbandry procedures, veterinary measures, financial situation of the owner, state subsidies, as well as the competence of the stockpersons (*Broom and Fraser, 2015; Hristov and Stanković, 2016; OIE, 2018a, b*). The basic principles of dairy cattle welfare plan creation and implementation was described by *Hristov et al. (2015a)* and some aspects of state of welfare on Serbian dairy farms by *Ostojić Andrić et al. (2016a)*.

Field experiences and scientific observations point to the need to define a guide which would describe general principles and good practices for ensuring the welfare of dairy cattle and which would be widely applicable. To do it, it is essential that the determination of the success of the implementation of good welfare practices on dairy cattle farms is based primarily on animal based measurements and to a certain extent on the provision of resources for the animals in the production system. This guide should identify key action areas in implementation the quality of a management system that takes into account the welfare of all categories of dairy cattle in the first place. The key action areas in the guide are stockmanship, feed and water, living environment, husbandry practices and health management. In each of these key action areas, there is a set of principles that should be used to further define the best good practice of securing dairy cattle welfare in farms (*Hristov and Stanković, 2016; IDF, 2019*).

The developed methods for the assessment of cattle welfare have contributed to the definition of good animal welfare practices on dairy cattle farms in form of the most important indicators of dairy cows welfare evaluation (*Hristov et al., 2012b*), their selection and implementation in on-farm assessment (*Ostojić Andrić et al., 2013*), relevance analysis and selection of key indicators for assessing the welfare of dairy cows (*Hristov et al., 2018*), different approaches to assess the welfare of dairy cows (*Hristov et al., 2014*) and assessment protocol for cattle (*Welfare Quality®, 2009*).

The aim of this paper is to describe the key action areas, namely their general principles, criteria and indicators in order to serve to implement good welfare practices in dairy cattle farms.

Dairy cattle welfare practices and stockmanship

The most important issue for the implementation of good welfare practices for dairy cows is stockmanship. The importance of good stockmanship and its benefits for the animals was described by *Rushen and Passille (2010)*. To assess the relationship of stockpersons to animals very important is the knowledge of the influences on the avoidance and approach behaviour of dairy cows towards humans, i.e., avoidance distance of cows in the stable, avoidance reactions in the feeding rack and approach behaviour towards an unfamiliar person as well as approach to a novel object (*Waiblinger et al., 2003*). It is well known fact that human factors (attitudes, personality, self-esteem and job satisfaction) strongly determine behaviour towards animals, animal production and animal welfare. Many studies have emphasised positive human contacts as indicators of a stockperson's positive attitude towards animals and animal welfare in general (*Boivin et al., 2003; Waiblinger et al., 2003; Broom and Fraser, 2015*). Some very important aspects of ethical stockmanship were given by *Hemsworth (2007)*. It is emphasized that housing and husbandry practices strongly affect farm animal welfare and thus stockpersons have a responsibility to provide specific standards of stockmanship for the animals. However, research suggests that the behaviour of some stockpersons is not as correct as it should be. Ethical discussion, using science and other considerations and involving stockpersons, livestock industries, government and the general public, should be used to establish and assure acceptable stockperson competencies across the livestock industries. Training programs targeting the key attitudes and behaviour of stockpersons presently offer the livestock industries good opportunities to improve human–animal interactions (*Boivin et al., 2003; Waiblinger et al., 2003; Hemsworth, 2007; Broom and Fraser, 2015; IDF, 2019*).

A good stockperson should have empathy for the animals concerned and competences to identify their needs and to provide them with the opportunity to satisfy them (*IDF, 2019*). Also, a good stockperson should have adequate knowledge of the needs and typical behaviour of all categories of dairy cattle (*Broom and Fraser, 2015*). Dairy cattle feeding should be fostered by positive relationships between stockpersons and animals, and in no case be reason for animal injury, panic behaviour, long-lasting fear and stress that can be avoided (*Hristov and Stanković, 2016*). Anyone who participates in different animal procedures as a stockperson, owner or other responsible person participating in a technological production process in any way should have developed competencies (knowledge, skills, attitude and other abilities), sufficiently to treat animals in accordance with the principles of welfare provision (*Broom and Fraser, 2015; IDF, 2019*). Principles of good welfare, have to consider essential to safeguard and

improve the well-being of farm animals are good feeding, good housing, good health and appropriate behaviour. Twelve clear criteria (absence of prolonged hunger, absence of prolonged thirst, comfort around resting, thermal comfort, ease of movement - other than health or resting-related issues, absence of disease - as well as neonatal and transport-related mortality, absence of pain induced by management procedures - including stunning, expression of social behaviours - balance between negative, e.g. prolonged and damaging aggression, and positive aspects, e.g. social licking, absence of injuries - except those due to disease or therapeutic or preventative interventions. expression of other welfare-related behaviours - balance between negative, e.g. stereotypies, and positive behaviours, e.g. exploration, good human-animal relationship - reduced fear of humans and positive emotional state) are also defined within the 4 principles (*Botreau et al., 2007*). It is particularly important that all participants in the technological process of dairy cattle production get acquainted with basic aspects described in guidance on risk assessment for animal welfare (*EFSA, 2012a*), outcome of the public consultation on the guidance on risk assessment for animal welfare (*EFSA, 2012d*), statement on the use of animal-based measures to assess the welfare of animals (*EFSA, 2012b*), scientific opinion on the use of animal-based measures to assess welfare of dairy cows (*EFSA, 2012c*), EFSA's internal project on the use of animal-based measures to assess animal welfare in EU (*EFSA, 2015a*), scientific report of EFSA on the effects of farming systems on dairy cow welfare and disease (*EFSA, 2009a*), scientific opinion on the risk assessment of the impact of housing, nutrition and feeding, management and genetic selection on metabolic and reproductive problems in dairy cows (*EFSA, 2009b*) and scientific opinion on the assessment of dairy cow welfare in small-scale farming systems (*EFSA, 2015b*).

Stockpersons, owners and any other persons in charge should be competent and well-trained, have experience and management skills that meet the level of technical requirements in the dairy cattle production system. In order to ensure the proper care of the animals, it is necessary to provide a sufficient number of employed persons for routine work, especially when it comes to peak activity. Veterinarians, animal husbandry engineers and other experts should be available continuously for advice on the animal care and should have supervisory capabilities. In order to monitor health and welfare, knowledge of the normal appearance and behaviour of all categories of dairy cattle is necessary. A competent stockperson should be able to understand the significance of changes in the behaviour of the animals. Also, it is necessary to know the early signs of distress or illness in order to be able to seek advice or intervention of a veterinarian in time. A competent stockperson should treat animals with compassion, anticipate potential problems and take the necessary preventive actions. Appropriate

equipment and instruments for the treatment of animals should be available (*Boivin et al., 2003; Broom and Fraser, 2015; Hristov and Stanković, 2016; IDF, 2019*).

Stockpersons who apply applying procedures under the control of animal husbandry engineers or veterinarian should demonstrate competence in particular for husbandry and veterinary procedures that can potentially cause animal pain and suffering, for example, the disbudding of young and dehorning adult animals, assistance in parturition and in the course of puerperium. Stockpersons should also have basic knowledge about the procedures with animals in loading, transport and unloading in accordance with national and international regulations. The competent body should define educational or training programs for stockpersons who should include acquiring basic knowledge of animal behaviour and best practices that ensure good animal welfare. In some developed countries and supply chains, there are quality assurance programs in place that relate to best animal welfare practices (*Main et al., 2014; Broom and Fraser, 2015; Hristov and Stanković, 2016; IDF, 2019*). Right now, these programs are not present in our country, but in any case, the stockpersons are required to be already familiar with the all relevant national regulations, and afterwards with standards and schemes for ensuring the welfare of animals related to the quality and safety of products as well as the welfare of animals (*Hristov et al., 2012a; Hristov and Stanković, 2016*). Anyway, keeping records should be ensured in order to demonstrate compliance with the quality assurance regulations and schemes (*Main et al., 2014*). In addition, it is necessary to continuously improve the technological process of production and in this way to prevent and correct the occurrence of the problems of dairy cattle welfare. Stockmanship can be generally improved by careful selection of stockpersons and by their training (*Boivin et al., 2003*). It is necessary to introduce systematic training of stockpersons in our country with special attention to stockmanship, feed and water, living environment, husbandry practices and health management in order to achieve a stable system of ensuring good dairy cattle welfare in the future (*Hristov and Stanković, 2016*).

Dairy cattle welfare practices and food and water

Appropriate nutrition is a basic requirement and it is considered to have a great role to ensure good dairy cattle welfare. Despite the importance of adequate water intake for both maintaining health and milk production, there has been very little research on drinking behaviour in dairy cattle. Stockpersons should be introduced to the feeding behaviour of dairy cows in order to consider improving cattle welfare and productivity (*Botheras, 2007*). They should keep in mind that inappropriate diets not only distort productivity, but also the health, behaviour and welfare of these animals. The animals must have access to enough food and water

that corresponds to age and needs to maintain normal health and productivity, to prevent prolonged hunger, thirst, malnutrition and dehydration. Provision of food and water should be in accordance with the physiological status of organism, lactation, pregnancy and growth, composition and quality of nutrients and climate conditions (Broom and Fraser, 2015). Milk production in adult and growing in young animals should be monitored and unexpected changes observed in time (IDF, 2019). Consideration should be taken into account that stocking density and feed barrier design affect the feeding and social behaviour of dairy cattle (Huzzey *et al.*, 2006). Stockpersons should get acquainted with body condition score and its relation with dairy cow productivity, health and welfare (Roche *et al.*, 2009).

Animals should have access to adequate amounts of appropriate food and water to maintain good health in order to ensure physiological and production requirements and reduce metabolic and nutritional disorders. It is essential to provide a balanced meal that ensures the metabolic needs of animals. Water supply must be in sufficient quantity and with adequate access to supplies, appropriate quality as well as regular water control and equipment maintenance. When it comes to young animals, they need to be provided with adequate food for growth (von Keyserlingk *et al.*, 2009; Broom and Fraser, 2015; Hristov and Stanković, 2016).

Basically, food and water must not contain any biological, chemical and physical substances harmful to health. Attention should be paid also to toxic plants and chemical substances as well as other harmful substances that can be ingested by animals. Wastewater effluents or chemical substances used for pest treatments on pastures and food must not get into the water or food in any case.

Food should be stored in a correct manner that prevents deterioration and ensures that contamination and deterioration in composition and quality does not occur at all. Changes in nutrition should be introduced gradually and accompanied by effects. The physical condition of animals should be monitored and evaluated in regular intervals, especially before and during parturition time, the peak of lactation and during drying period. Appropriate minimum levels of body condition should be defined, below which urgent remedial actions are undertaken along with veterinary advice. It is also necessary to take into account the presence of fat cows in the herd (Roche *et al.*, 2009; Hristov and Stanković, 2016). Nutrition and water supply systems should be controlled in terms of functioning and any problems that arise in time need to be addressed. Where possible, alarms indicating the occurrence of the problem should be installed. There should be a system that works in case of emergency. Special attention should be given to the additions of minerals and vitamins to maintain good health in any case with advice from professionals who know potential deficits or surpluses of micronutrient in a particular geographic area (Hristov and Stanković, 2016; IDF, 2019).

Nutrition and water supply equipment should be located in such a way that animals can consume food and water unhindered, and in doing so may be able to express normal behaviour, and in particular to reduce the risk of agonistic behaviour expression. The number of animals per feeder and drinkers unit, as well as pasture areas and food and feed space, should be assessed and communicated in each case (*Huzzey et al., 2006*). This equipment should also be properly designed and constructed to provide sufficient amount of food and water, and in any case to avoid contamination and injuries to animals themselves when consuming food and water. When living on pastures, care should be taken to ensure that there is enough food and water to avoid starvation and thirst, especially during the drought periods of the year when it is often necessary to provide additional food (*Hristov and Stanković, 2016*).

When it comes to young animals, adequate colostrum should be provided, in terms of quality and quantity, and in the case that it is not provided on the farm, adequate replacement of the commercial colostrum (*Relić, et al., 2014*). The first colostrum should be consumed as soon as possible and at the latest six hours after birth. In situations where calves do not allow sucking in pre-weaning period, they should receive liquid food in a way that allows for sucking needs (for example, through teats bucket or the like). Calves during the sucking period should not be deprived of liquid food until the rumen develops sufficiently to use solid nutrients and thus meet the nutritional needs. During the sucking period, especially the newborn calves, should be milk fed at least twice a day to ensure sufficient consumption. Weaned replacement heifers, for further use for reproductive purposes, should have access to balanced, solid foods of good quality from the early age to accelerate the corresponding development of the rumen. Cud feeding can be a good alternative for the development of the physiology of the rumen in weaned replacement heifers that serve as a substitute in reproduction (*IDF, 2019*). Changes in feeding, drinking, and standing behaviour of dairy cows during the transition period should be carefully considered (*Huzzey et al., 2005*). All equipment for feeding young animals should be thoroughly cleaned after use. Food and pastures should be controlled in terms of quality and quantity (*Broom and Fraser, 2015; IDF, 2019*).

Dairy cattle welfare practices and environment

Environment, space, equipment, microclimatic and hygienic conditions significantly influence the welfare of dairy cattle. In our country, there are literature data on relations between rearing conditions, health and welfare of dairy cows were described (*Hristov et al., 2008*), minimum standards in conditions of rearing and welfare of cattle (*Hristov et al., 2007*), influence of rearing conditions

on calf welfare in the first month of life (*Samolovac et al., 2019*), methods of assessment of the conditions of breeding and welfare of dairy cows (*Maksimović et al., 2007*), assessment welfare of cows in free housing (*Hristov et al., 2011*) as well as standards of welfare and biosecurity on cattle farms (*Hristov et al., 2009*) so stockpersons can use them in everyday practice. Also, relation between housing conditions and welfare of dairy cows in Serbia was described by *Ostojić Andrić et al. (2015)*.

Primarily, the adequate physical, thermal and psychic comfort of the animals should be ensured. Particular attention should be paid to ensure proper milking parlours and handling yards, shelter and appropriate conditions in the stalls. A detailed plan should be defined for the evacuation of animals in case of fire, earthquake or floods (*Broom and Fraser, 2015; Hristov and Stanković, 2015; IDF, 2019*).

The environment should be designed, constructed and maintained so that it can meet the needs of dairy cattle. Particular attention should be paid to the implementation of activities related to all categories of the animals. Requirements to be provided in the environment, depend on physical and climatic conditions as well as the management system. When it comes to the physical aspects of the environment, special attention should be paid to lying surfaces, feedlots areas and yards, bedding, ventilation, fences and regular removal of manure. Stockpersons should have knowledge about behavioural indicators of cows' comfort, especially active and resting behaviour of dairy cows (*Haley et al., 2000*). Stockpersons should keep in mind that overstocking reduces lying time in dairy cows (*Fregonesi et al., 2007*). Also, grouping and social preferences in calves, heifers and cows should be considered (*Bøe and Færevik (2003)*).

Regarding climate factors, special attention should be paid to ensuring temperature, humidity, air flow rates, lighting, low levels of noise and vibration. Hazardous gases in the stalls should be kept in stalls. Social grouping of animals is very important for their well-being. Special attention should be paid to grouping calves in group pens. Animals must not be permanently tethered. When keeping animals in confined spaces, especially newborns in individual boxes, calves should be enabled to lie down, stand up, turn around, rest, and maintain a normal body position and body care without difficulty (*Broom and Fraser, 2015; Hristov and Stanković, 2015; IDF, 2019*).

Dairy cattle welfare practices, husbandry and veterinary procedures

Dairy cattle should be treated with care and in a consistent manner, taking into account their natural behaviour all the time, minimizing the risk of injury and

distress. In the procedures, special attention should be given to moving the animals from one environment to another, grouping the animals, applying the electric prodders, fixing animals, marking, dehorning, shortening the tail, correcting the hoofs, loading, transporting and unloading animals, veterinary treatments and assisting in parturition. In all these cases care should be taken to avoid causing unnecessary pain, fear and consequently animal suffering (*Broom and Fraser, 2015; Hristov and Stanković, 2016, IDF, 2019*).

In the paper of *Vasseur et al., (2010)* some risks factors in term of welfare have been identified. They include: low use of a dedicated calving pen and infrequent surveillance of calving, no disinfection of newborn's navel and delayed identification and, hence, delayed calf monitoring, risks relying on suckling as a source of colostrum, or delaying and providing insufficient quantities, and unchecked immunoglobulin quality and immunity transfer, dehorning and removing supernumerary teats at late age and without pain control, waste milk given without precaution and traditional restrictive feeding of milk or substitute, weaning targeted on age rather than on concentrate intake, and calves housed individually and in inappropriate housing systems. All these risk factors stockpersons should keep in mind and continuously consider the introduction of good practices in calf management since ensuring welfare is very important in rearing of newborn animals (*Relić, et al., 2014; Broom and Fraser, 2015; Hristov and Stanković, 2015*).

Dairy cattle welfare practices and health condition

For the animals' welfare, health conditions have particular importance, such as: lameness, mastitis, injuries, acidosis, milk fever and other metabolic diseases, diarrhoea and anaemia of calves, respiratory diseases, heat stress, arthritis and contagious diseases (*IDF; 2019*). Welfare and behaviour in relation to disease of dairy cows were described by *Hristov et al. (2015b)*. Scientific opinion on the risk assessment of the impact of housing, nutrition and feeding, management and genetic selection on metabolic and reproductive problems in dairy cows can be found in *EFSA (2009b)*. Scientific report of *EFSA (2009a)* provides insights into numerous researches on the effects of farming systems on dairy cow welfare and disease. There are numerous papers in our country that deal with behaviour of cattle as an indicator of their health and welfare (*Relić et al., 2012*), key health issues affecting dairy cows welfare (*Ostojić Andrić et al., 2016a*), welfare and behaviour in relation to disease of dairy cows (*Hristov, 2015b*), health and welfare of dairy cows (*Ostojić Andrić et al., 2016c*), dairy cows health parameters in different season - a welfare approach (*Ostojić Andrić et al., 2017*), frequency of behavioural disorders of calves in the first month of life (*Samolovac et al., 2018*).

and the most common health disorders and welfare of dairy cows and calves (Stanković, 2014).

In relation to these conditions, advice can be obtained from veterinary experts. Treating dairy cattle by unqualified staff may lead to serious health and welfare problems, which should not be allowed in any case. This can be particularly the case with an inappropriate diagnosis, poor surgical interventions with incorrect and inadequate analgesia and anaesthesia. Farm management plans in place should be in line with relevant national and international veterinary requirements. Viral, bacterial and parasitic diseases should be prevented and controlled through appropriate biosecurity measures, appropriate technological process of production and good management practices including prophylaxis and regularly monitoring. Separate facilities should be provided for diseased and injured animals (Broom and Fraser, 2015; Hristov and Stanković, 2015; IDF, 2019).

When it comes to health, stockpersons should have appropriate competencies in terms of identifying the first signs of illness and injury in dairy cattle, as well as the implementation of appropriate standard operational procedures for the health management in relation to the animal welfare in cooperation with the veterinarian. Particular attention should be paid to the daily inspection of animals, especially those who are around the parturition period, newborns and close weaned animals, animals kept in confined areas, animals affected by metabolic disorders, emergencies, outbreaks of contagious diseases, and where economic and psychosocial problems related to dairy cattle exist (Broom and Fraser, 2015; Hristov and Stanković, 2015; IDF, 2019).

The animal health programs should take into account preventive measures against abovementioned diseases and injuries, mineral and vitamin supplements in order to prevent deficiencies, supplementation of magnesium and calcium around the parturition period, hygiene of the animals, barns, shelters, loafing areas and yards, pastures and milking parlours, proper nutrition, management of ambulatory animals as well as isolation and prompt treatment of diseased animals. The stockman is expected to implement a detailed biosecurity plan to reduce the risk of introducing and spreading disease on the farm. It is also necessary to introduce a system of records data related to the health protection plan, such as animal identification, morbidities rates, culling rates, reproductive disorders, lameness, incidence of mastitis, incidence and details of preventable diseases and injuries, vaccinations, diagnostic tests and all treatments with withdrawal times for medicines whether controlled by veterinary regulations or not. All aspects related to euthanasia when necessary in diseased or sick animals should also be considered (Broom and Fraser, 2015; Hristov and Stanković, 2015; IDF, 2019).

Conclusions

On the basis of the presented data from the literature and the experience of the authors of the paper related general principles and good animal welfare practice on dairy cattle farms can be concluded:

- it is necessary to define a guide which would describe the general principles and good practices for ensuring the welfare of dairy cattle;
- the key action areas in the guide should be stockmanship, feed and water, living environment, husbandry practices and health management;
- the most important issue for the implementation of good welfare practices for dairy cows is stockmanship;
- it is necessary to introduce systematic training of stockpersons in our country with special attention to stockmanship, feed and water, living environment, husbandry practices and health management in order to achieve a stable system of ensuring good dairy cattle welfare.

Opšti principi i dobre prakse za dobrobit životinja na farmama mlečnih goveda

Slavča Hristov, Dušica Ostojić Andrić, Branislav Stanković

Rezime

Iskustva na terenu i naučna zapažanja ukazuju na potrebu definisanja vodiča u kome bi se opisali opšti principi i dobre prakse za obezbeđenje dobrobiti mlečnih goveda koje bi bile široko primenljive. Ključne oblasti u vodiču treba da budu odgajivanje, hrana i voda, životna sredina, zootehnički i veterinarski postupci i upravljanje zdravljem životinja. U svakoj od ovih ključnih oblasti postoji skup principa koji bi trebalo da se koriste za dalje definisanje najboljih dobrih praksi za obezbeđenje dobrobiti mlečnih goveda na farmama. Najvažnija oblast za implementaciju dobrih praksi dobrobiti mlečnih krava je odgajivanje. Odgovarajuća ishrana je osnovni zahtev i smatra se da ima veliku ulogu u obezbeđenju odgovarajuće dobrobiti mlečnih goveda. Okolina, prostor, oprema, mikroklimatski i higijenski uslovi značajno utiču na dobrobit mlečnih goveda. Mlečna goveda treba tretirati pažljivo i na konzistentan način, uzimajući u obzir njihovo prirodno ponašanje sve vreme, a u svakom slučaju umanjiti rizik od povreda i stresa. Za dobrobit mlečnih goveda posebno su važna zdravstvena stanja, kao što su: šepavost, mastitis, povrede, acidoza, mlečna groznica i druga metabolička oboljenja, dijareja i anemija teladi, respiratorne bolesti, toplotni stres,

arthritis i mnoge zarazne bolesti. Neophodno je uvesti sistematsku obuku odgajivača u našoj zemlji sa posebnim osvrtom na odgajivanje, hranu i vodu, životnu sredinu, zootehničke i veterinarske postupke i upravljanje zdravljem, kako bi se dostigao stabilan sistem obezbeđenja odgovarajuće dobrobiti mlečnih goveda.

Ključne reči: dobrobit mlečnih krava, opšti principi, dobre prakse dobrobiti

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EMOTIONAL STATE OF DAIRY COWS IN LOOSE AND TIED HOUSING SYSTEM - IS THERE A DIFFERENCE?

Dušica Ostojić Andrić¹, Slavča Hristov², Radica Đedović², Teodora Popova³, Vlada Pantelić¹, Dragan Nikšić¹, Nenad Mičić¹

¹Institute for Animal Husbandry, Autoput 16, 11080 Belgrade-Zemun, Serbia

²Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Belgrade-Zemun, Serbia

³Institute of Animal Science, Pochivka str., 2232 Kostinbrod-Sofia, Bulgaria

Corresponding author: Dušica Ostojić Andrić, andricdusica.iah@gmail.com

Invited paper

Abstract: Modern society, over time, has accepted animals as sentient beings. From the welfare point of view, the emotions are a reflection of the whole permeation of the animal and its environment, which make them important and reliable indicators. In fact, they are cross-linked with other welfare indicators such as physiological and behavioural. Freedom of movement is crucial for the animals' naturalness, especially in terms of their normal behaviour expression. Regarding that, this study was conducted in order to investigate and compare the emotional state of cows in a loose (LHS) and tied housing systems (THS). The assessment was done according to Welfare Quality Protocol for Cattle (2009) on a total of 16 dairy farms (N=4,833 cows), seven with LHS and nine with THS. The emotional state was assessed by a qualitative behavioural assessment (QBA), a method based on the translation of "body language" into the numbers that can be statistically analysed and interpreted. Twenty reliable descriptors were used, among which, some were related to positive emotional state (e.g., active, relax, happy) and some to negative state (e.g., agitated, nervous, distressed). Result showed that tendencies toward positive descriptors were significantly higher ($p \leq 0.05$) in LHS, followed by lower expression of distress, frustration and apathy in animals. This resulted in better estimation of overall emotional state in LHS indicating its advantage and necessity of providing exercise in cows' daily routine.

Key words: dairy cows, emotional state, QBA, descriptors, welfare

Introduction

The welfare of animals is closely related to their emotional state, i.e. feelings they experience, which can be positive (happiness, curiosity, friendliness, etc.) or negative (fear, frustration, pain, etc.). Feelings motivate animals to express

their needs (e.g. the need for food and water is expressed by the feeling of hunger and thirst while loneliness is associated with a lack of social contact). This points to a strong and inseparable connection between feelings and welfare that is essentially based on meeting the needs of animals. However, in modern conditions of breeding and management of dairy cows, there are often situations in which their needs actually deviate from the feelings. Such is the case with highly productive cows, which, despite their abundant diet, often exhibit a sense of hunger (Roche, 2009). They can be in deep conflict because of the constant feeling of hunger and the need to feel full on the one hand and, on the other hand, the need to do something else, like chewing or relaxing. Such a cow Webster (2005) describes in the following way: "her feelings can be described with brutal precision as a constant hunger, fatigue, overflow and nausea". There are many examples where negative feelings of high intensity or long duration result in animal suffering. Such is the case with feelings of fear, illness, fatigue, anxieties, boredom, depression, sadness, paranoia, agony, etc. (Gregory, 2004). Every pathological state of the organism and the accompanying feelings reduces the production potential of an animal with a normal physiological status indicating that the feelings are also of influence to the productivity.

In the study by Duncan (2005), the assessment of positive and negative emotional states as a welfare indicator is of great importance. Possible indicators of positive emotional state include social forms of behaviour: licking, exploratory behaviour and play, while fear and anxiety are indicators of a negative emotional state.

In assessing the emotional state of animals, however, the question is how to quantify the feelings? It was noted by many authors (Boissy *et al.*, 2007; Wemelsfelder *et al.*, 2009) that the animal's body language represents a matter of fact its behavioural expression and can reveal important aspects of its physical and mental health, and therefore welfare. The methodological approach that translates body language into figures that can then be statistically processed and interpreted is called a qualitative behavioural assessment (QBA). By evaluating the QBA, we obtain information on the overall response of the animal to the conditions that are provided to it in certain environment, so the QBA measures the "outcomes" and contributes to the assessment of welfare, as it can include variations in the way animals react and deal with their environment at certain moment in time (Fleming *et al.*, 2016). The significance of the QBA assessment is also reflected in the fact that the research found its significant correlation with physiological welfare indicators (Hemsworth and Barnett, 2001; Stockman *et al.*, 2011, 2013). Animals assessed to be highly anxious, upset, or nervous had elevated neutrophil : lymphocyte ratio or higher plasma lactate concentration, which are typical stress markers (Jones and Allison, 2007).

Although, QBA assesses the whole animal and QBA scores are correlated with physiological condition and behaviour it is not known yet for sure if it could be taken as a stand-alone tool in welfare assessment. In support of this suspicion goes recent study of *Andreasson et al., 2013* who did not find correlations between QBA and other welfare indicators estimated by *Welfare Quality® Protocol (2009)*.

However, taking into account all mentioned, it is clear that the emotional status of animals plays an important role in assessing the state of welfare and that it probably best reflects the degree of its vulnerability. Starting from the concept of naturalness (the ability for an animal to live a reasonably natural life), freedom of movement can be considered one of the most important preconditions for ensuring a positive emotional state (*Boogaard et al., 2011*). The cows that are kept tied are, among other things, deprived of the opportunity to investigate their environment and have natural social contacts. In addition, numerous studies have shown that disorders and diseases occur more frequently in tied systems, and are also considered to be the source of animal suffering (*Regula et al., 2004; EFSA, 2009; Ostojić Andrić et al., 2011*). Therefore, the aim of this work was to determine and compare the emotional state of the cows in the loose and tied housing system using the QBA assessment.

Materials and Methods

The study was conducted on 16 selected commercial dairy farms (Mean \pm SEM, 301 \pm 71.6 lactating cows), 9 with tie-stall housing systems -THS (Mean \pm SEM, 266 \pm 99.6 lactating cows) and 7 with loose housing system-LHS (Mean \pm SEM, 348 \pm 104.7 lactating cows), in Serbia. The cows had access to outdoor loafing area in 4 of 9 tie-stall farms and pasture only on one farm (24 hours a day for 60 days a year). Each farm in this study was visited twice a year, in the winter and summer season, and the average value of each welfare measure was calculated. Three trained assessors evaluated the cows on each farm. Prior to each farm assessment, the agreement with animal unit's manager was made in order to avoid disturbing of usual farm activities.

The QBA was made using the method described by the *Welfare Quality® Assessment Protocol for Cattle (2009)* where detailed information about the methodology can be found. QBA relies on observer assessments of the body language of animals using a set of twenty descriptive terms - descriptors: active, frustrated, irritable, relaxed, friendly, uneasy, fearful, bored, sociable, agitated, playful, apathetic, calm, positively occupied, content, lively, distressed, indifferent, inquisitive and happy. These terms were estimated (0 - 125 mm) according to Visual analogue scale (VAS) and by specific weight coefficients and I-spline

functions translated into score (0-100 points) by Welfare Quality® Scoring System Software Program (2016) resulting in the final estimate of the emotional state of cows in the loose and tied system.

Statistical analyses were performed using Statistica for Windows version 8.0 (StatSoft, Inc. 2010, data analysis software system). The statistical significance of the effect of housing system on cows' emotional state was determined by the t-test or the Mann-Whitney test, depending on the normal or abnormal distribution of the data, established with the Kolmogorov-Smirnov test. P values less than 0.05 were considered as significant.

Results and Discussion

The results of the research presented in Table 1 show that the emotional state of cows is significantly better assessed in the loose housing system ($p \leq 0.05$). On the basis of the obtained score of 60.4 points, the emotional state of cows on LHS farms can be described as enhanced while for the THS farms it belongs to a lower, acceptable category (43.2 points). For the positive descriptors of QBA, happy and positively occupied, the average score was significantly higher ($p \leq 0.05$) in LHS, while for negative descriptors such as frustrated, apathetic and distressed the value was significantly higher ($p \leq 0.05$) in THS.

Similar results can be found in studies by other authors who however, have found statistical differences in a number of descriptors. Thus, study of *Vučemilo et al. (2012)* shows that cows in tied systems with occasional mobility also have higher values of positive descriptors (happy, positively occupied, active, relaxed and sociable) than those who are continuously tied. In line with this, *Popescu et al. (2014)* lists statistically significantly higher values of negative descriptors (agitated, distressed, frustrated, indifferent, bored, irritable, uneasy, etc.) in cows kept tied.

Table 1. shows that in both housing systems, the medium to high value of positive descriptors has been determined, especially for terms active, relaxed, calm, content, friendly, lively, sociable (≥ 50 mm) which corresponds to an acceptable assessment of the emotional state in the investigated farms. In a research by *Popescu et al. (2013)*, negative descriptors were prevalent in both examined hold systems, which affected the poor QBA values as determined here. However, as in our research, the value of QBA was better evaluated in a housing system that provided greater mobility.

Table 1. Estimation of overall emotional state, QBA and descriptors (VAS) in LHS and THS

Housing system	LHS					THS					F
Score for emotional state, points	\bar{x}	SD	S^2	Min	Max	\bar{x}	SD	S^2	Min	Max	
	60.40	13.43	180.40	32.30	89.30	43.23	22.85	522.05	10.20	92.70	*
Descriptors, VAS (0-125mm)											
Active	78.69	19.09	0364.26	48.68	118.00	64.99	24.28	589.68	36.41	109.00	ns
Relaxed	82.47	12.07	145.75	63.11	105.00	73.45	17.25	297.43	30.34	113.50	ns
Fearful	2.22	2.67	7.14	0.00	8.72	1.46	2.81	7.88	0.00	11.97	ns
Agitated	2.13	2.91	8.45	0.00	8.15	5.47	9.12	83.18	0.00	37.23	ns
Calm	75.96	12.34	152.27	59.47	98.50	67.43	18.70	349.63	24.27	102.10	ns
Content	71.80	15.55	241.90	50.97	110.00	66.40	21.06	443.40	31.55	115.80	ns
Indifferent	13.91	11.04	121.82	0.00	42.11	8.03	7.55	57.03	0.00	25.00	ns
Frustrated	13.28	10.69	114.19	0.00	31.50	38.80	28.98	839.91	0.00	78.95	**
Friendly	66.33	22.55	508.72	29.07	102.63	75.10	16.00	255.88	51.32	101.90	ns
Bored	34.96	20.99	440.55	2.70	67.11	43.00	25.31	640.68	6.58	84.21	ns
Playful	42.81	17.11	292.59	12.10	71.30	32.64	17.40	302.78	5.26	77.45	ns
Positively occupied	78.46	16.77	281.22	41.60	110.40	60.46	21.62	467.53	37.62	110.70	*
Lively	58.75	19.33	373.78	6.25	85.30	56.35	19.00	360.86	28.95	96.47	ns
Inquisitive	72.35	25.28	638.89	23.26	98.68	89.34	14.74	217.26	55.26	107.89	*
Irritable	9.57	12.06	145.44	0.00	48.54	11.59	12.74	162.42	0.00	43.69	ns
Uneasy	2.74	4.51	20.32	0.00	17.44	5.71	7.69	59.13	0.00	26.60	ns
Sociable	68.87	22.24	494.42	31.20	119.10	68.98	18.70	349.74	40.50	97.10	ns
Apathetic	2.58	5.04	25.40	0.00	18.20	15.25	22.65	513.03	0.00	78.30	*
Happy	59.05	11.70	136.94	41.80	85.60	47.11	16.90	285.71	22.50	91.40	*
Distressed	27.62	11.31	127.81	5.30	43.69	59.95	26.74	715.08	2.50	93.45	**
QBA	0.95	1.44	2.08	-2.20	4.21	-1.07	2.74	7.53	-5.72	4.72	*

ns = p>0.05; * = p<0.05 ; ** = p<0.01

Table 1 shows that in both housing systems, the medium to high value of positive descriptors has been determined, especially for terms active, relaxed, calm, content, friendly, lively, sociable (≥ 50 mm) which corresponds to the acceptable assessment of the emotional state in the investigated farms. In a research by *Popescu et al. (2013)*, negative descriptors were prevalent in both examined housing systems, which affected the poor QBA values than values obtained in the present study. However, as in our research, the value of QBA was better evaluated in the housing system that provided greater mobility.

It is interesting that in mentioned studies, including the present one, the descriptor inquisitive was significantly higher in tied cows. Explaining this, we can refer to the findings of *Krohn (1994)* who states that increased expression of exploratory behavior in THS has a character of curiosity that can be explained by insufficiently stimulating environmental conditions and lack of social contact.

Conclusion

Animal welfare includes both physical and mental aspects of an animal's experience, and therefore both physiological and behavioural indicators are useful in assessment. The emotional state of an animal is a comprehensive outcome that probably best reflects the endanger of its welfare. Although it is recommended to include other welfare indicators in assessment, QBA can potentially be used as a quick, 'first pass' screening method to identify farms in risk and take the further in-depth assessment. Given this, the results of our study have shown that the emotional state of cows in LHS is characterized as enhanced, unlike THS in which the emotional needs of cows were provided at a minimal level, with special concern to distress and apathy.

In general, it can be concluded that in order to provide a positive emotional state in cows, their daily routine must be enriched by exercising in paddock, pasture or any other movement in the environment that enables expression of natural forms of behavior.

Emocionalno stanje mlečnih krava u slobodnom i vezanom sistemu držanja – postoji li razlika?

Dušica Ostojić Andrić, Slavča Hristov, Radica Đedović, Teodora Popova, Vlada Pantelić, Dragan Nikšić, Nenad Mičić

Rezime

Savremeno društvo vremenom je prihvatilo životinje kao osećajna bića. Sa stanovišta dobrobiti, emocije su odraz celokupnog prožimanja životinje i njene sredine, što ih čini važnim i pouzdanim pokazateljima. One su, zapravo povezane sa drugim pokazateljima dobrobiti kao što su fiziološki i bihejvioralni. Sloboda kretanja je ključna za prirodni život životinja, posebno u smislu mogućnosti

izražavanja prirodnih oblika ponašanja. Sa obzirom na to, ova studija sprovedena je da bi se istražilo i uporedilo emocionalno stanje krava u slobodnom (LHS) i vezanom sistemu držanja (THS). Procena je obavljena prema Welfare Quality Protocol for Cattle (2009) na ukupno 16 mlečnih farmi (N=4,833 krava), sedam sa slobodnim i devet sa vezanim sistemom držanja. Emocionalno stanje procenjeno je kvalitativnom ocenom ponašanja (QBA), metodom koja se zasniva na prevodenju “govora tela” u brojeve koji se mogu statistički analizirati i interpretirati. Korišćeno je dvadeset pouzdanih deskriptora, od kojih se neki odnose na pozitivno (npr. aktivnost, opuštenost, sreća), a neki na negativno emocionalno stanje (npr. uznemirenost, nervoza, distres). Rezultati su pokazali da su tendencije prema pozitivnim deskriptorima bile značajno veće ($p \leq 0,05$) u LHS-u, pri manjoj ekspresiji distresa, frustracije i apatije kod životinja. To je rezultovalo boljom procenom ukupnog emocionalnog stanja u LHS-u, ukazujući na njegovu prednost kao i potrebu da se kravama omogući svakodnevno kretanje pri gajenju.

Ključne riječi: mlečne krave, emocionalno stanje, QBA, deskriptori, dobrobit

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EFFECT OF STARTING MILK FLOW ON UDDER HEALTH OF HOLSTEIN COWS

Pero Mijić¹, Tina Bobić¹, Mirjana Baban¹, Maja Gregić¹, Franjo Poljak², Vesna Gantner¹

¹Faculty of Agrobiotechnical Sciences Osijek, Josip Juraj Strossmayer University of Osijek, 31000 Osijek, Croatia

²Croatian Agency for Agriculture and Food, Vinkovačka cesta 63c, 31000 Osijek, Croatia

Corresponding author: Pero Mijić, pmijic@fazos.hr

Invited paper

Abstract: Selection of cows for high milk production resulted in reduced resistance of animals to various diseases such as udder inflammation, which is also a consequence of udder maladjustments for mechanical milking. Studying of milk flow and application of modern measurement devices could lead to higher number of cows adjusted to mechanical milking and reduce udder disease risks. Milk flow in mechanical milking of cows consists of several different phases that can differently affect not only the length of milking, but also the udder health. The aim of the research was to evaluate effects of starting milk flow (tS500) on udder health. The research was carried out on 457 Holstein cows. Udder health (logarithmic somatic cell count - LSCC) was at the greatest risk in cows being in the third lactation (LSCC=3.17 ± 0.12). Starting milk flow (tS500) was equable through all lactations, being the longest in cows in the second lactation (0.21 ± 0.01 min). The least effect on udder health (LSCC=2.38 ± 0.23) when tS500 was comprised between 0.31 and 0.40 min. Longer as well as shorter duration of starting milk flow resulted in significantly higher number of somatic cells in milk of Holstein cows.

Key words: starting milk flow, udder health, Holstein cows

Introduction

Selection of cows for high milk production resulted in reduced resistance of animals to various diseases such as udder inflammation, which is also a consequence of udder maladjustments for mechanical milking (*Rupp and Boichard, 1999*). Environmental regulations imposed by the European Union to demand that healthy animals and their products can come only through strict hygienic and

sanitary norms, and pharmaceuticals and veterinary services must reduce their use of drugs (EEA, 2010). Right here is the opportunity to work through the selection choices made with such cattle milking traits that would suit both the requirements of milking equipment, and the requirements for good udder health (Graff, 2006). Because of that, the milkability traits of dairy cows are studying more in breeding-selection work. The Politiek (1961) are describe milkability traits on the best way: Milkability is capability of cows to have enough fast milking time, to be milked: evenly, completely, and in the same time from all quarters of the udder. Should be kept in mind that milking of the cows takes 50% of working time on dairy farms, which make a milking process extremely important in the organization of the staff (Rotz *et al.*, 2002).

Studying of milk flow and application of modern measurement devices could lead to higher number of cows adjusted to mechanical milking and reduce udder disease risks (Roth *et al.*, 1998). Milking of cows can be improved by standardizing cow udders through selection and by applying new techniques (Mijić *et al.*, 2003). For the udder health of the cow's, milking should take a short time, the maximum flow of milk should be reached quickly and keep it for as long as possible. However, some problems arise because the dough properties are subject to the influence of various environmental factors. Milk flow in mechanical milking consists of several different phases, and their relation to udder health has become scientifically interesting (Sandrucci *et al.*, 2005).

Before every milking, it is necessary to make certain preparing actions on the udder and teats, respectively. This actions implied washing and cleaning of the teats from impurities and their disinfection. During the course of these actions, the pituitary gland begins to secrete the hormone oxytocin which is extremely important for milk releasing from the udder. This kind of prepared cows will have proper and high quality milking. In the absence of these preparatory actions, at the beginning of the milking (start milk flow) the appearance of, so called, bimodal milk flow (initial sudden climb, and after that sudden drop) (Mijić *et al.*, 2005). The hypothesis of this paper is that appearance of the bimodal milk flow influence on the milking duration and on the udder health. The aim of the study was to measured milk flow of the cows of Holstein breed, and also determine the health status of the udder depending of the different values of the starting milk flow.

Materials and Methods

Experimental animals

The research was carried out on a dairy in Eastern Croatia, on 457 cows of Holstein breed. Electronic mobile measurement device for cow milk productivity control,

called Lactocorder®, was used in the research. All animals involved in the experiment had correct morphologic udder traits and were not treated with medicaments against mastitis. Following parameters were used: milk yield (MY), average milking speed during the main milking process (DMHG), maximum milk flow rate (HMF), time to reach 0.5 kg/min at beginning (tS500), time of increasing phase (tAN), time of plateau phase (tPL), time of decline phase (tAB) and bimodality (BIMO). The bimodality is a parameter in the increase phase. The stimulated double peak progression at the start of milking is recorded by it. Graphic scheme of milk flow curves with different stages is shown in Figure 1.

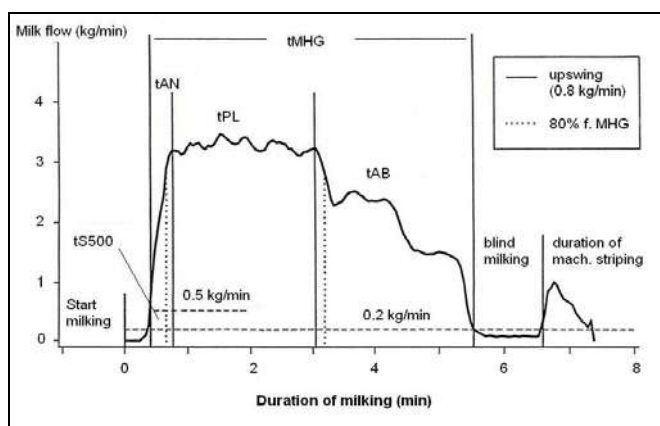


Figure 1. Graphic scheme of the milk flow curve with individual phases (Göft, 1992)

The milking equipment was checked before every milking and coordinated with the same under pressure values (43-48 kPa), pulsation relations (1:1) and tact numbers (58-60). For each cow one measuring was performed in the period of the 50th to 180th lactation day, whereby the German regulations (*ADR, 1987*) were used. The milk samples taken by LactoCorder were analysed in the Central State Laboratory for milk quality control in Križevci (Croatia) whereby the somatic cell count was established by Fossomatic 5000.

Data Analysis

Cause of even distribution mean number of somatic cells in milk (SCC), for all analyzed milk samples a linear transformation was performed using the formula (*Dodenhoff et al., 1999*):

$$(\log_2 (\text{SCC}/100.000) + 3)$$

and marked as LSCC. The data collected from this study was subjected to analysis of variance (ANOVA) using StatSoft, ver. 8.1. Data analysis was done for the lactation (1, 2 and 3) and the duration of starting milk flow tS500 (≤ 0.10 , 0.11-0.20, 0.21-0.30, 0.31-0.40 and ≥ 41 min.), which refers to the time that passes from the start of milking until milk flow reached 0.5 kg/min. Multiple comparison of mean values was made with the Post Hoc Test together with Fisher LSD method at the significant level of $P < 0.05$ and $P < 0.01$.

Results and Discussion

Milk production per milking increased considerably through lactation periods, being on average 10.18 ± 0.29 kg (Table 1). Referring to our research results, maximum milk flow was increased (from 3.22 to 3.74 kg/min) proportionally to milk productivity increase, which agrees with the results of the Bobić et al., (2013). The tS500 was equable through all examined lactations, being the longest in the second lactation (0.21 ± 0.01 min). Bobić et al. (2013) are determined a negative correlation ($r^2 =$ from -0.21 to - 0.47) between tS500, tPL, tAB, HMF and MHG. The parity significantly affects the milkability, milk and health traits such as somatic cell count (Bobić et al., 2017), which is confirmed in this research, namely, occurrence of bimodality was observed, being the most intense in the first lactation (46.50 ± 0.50 %), and the highest LSCC was determined in cows in the third lactation (3.17 ± 0.12). Among other milk flow phases, the longest duration was determined for the decline phase (2.14 ± 0.10 min), which was very unfavorable in terms of selection. Such result is an indicator of uneven udder quarters that prolong milking duration and expose empty udder quarters to irritation of milking device. Because of that, every part of the milking is crucial which influence on the quality of milking and udder health. The results from Germany are shown that percent of the culling cows because of the udder health, range between 15.5 to 20.3%. Besides that, the many subclinical illness' animals are present, which results in significant financial losses in milk production (Roth et al, 1998).

Table 1. Milk flow parameters and somatic cell by lactation (Least square means \pm Standard error of means)

Trait	Unit	Lactation			Total (n=457)
		1. (n=157)	2. (n=113)	3. (n=187)	
MY	kg/milking	9.22 \pm 0.23 ^A	9.84 \pm 0.34 ^A	11.18 \pm 0.29 ^B	10.18 \pm 0.29
MHG	kg/min	2.12 \pm 0.07 ^A	2.43 \pm 0.08 ^a	2.57 \pm 0.08 ^b	2.45 \pm 0.07
HMF	kg/min	3.22 \pm 0.09 ^A	3.45 \pm 0.11 ^a	3.74 \pm 0.11 ^{Bb}	3.49 \pm 0.10
tS500	min	0.19 \pm 0.01	0.21 \pm 0.01	0.20 \pm 0.01	0.20 \pm 0.01
tAN	min	0.70 \pm 0.04	0.84 \pm 0.06	0.74 \pm 0.04	0.75 \pm 0.05
tPL	min	1.71 \pm 0.11	1.71 \pm 0.14	1.63 \pm 0.09	1.67 \pm 0.12
tAB	min	2.09 \pm 0.11	2.02 \pm 0.11	2.24 \pm 0.08	2.14 \pm 0.10
BIMO	%	46.50 \pm 0.50 ^a	38.94 \pm 0.49	34.22 \pm 0.47 ^b	39.61 \pm 0.49
LSCC	log	2.72 \pm 0.13 ^a	2.90 \pm 0.16	3.17 \pm 0.12 ^b	2.14 \pm 0.13

For Tables 1. and 2. ^{a, b, c} P<0.05; ^{A, B, C} P<0.01

Highest number of somatic cells in milk (LSCC=3.06 and 3.37) was determined when tS500 lasted up to 0.20 min and longer than 0.41 min (Table 2). There were 45.3% such cows of total number involved in the research. Duration of tS500 from 0.31 to 0.40 min had the least effect on udder health (LS=2.38). Obtained results referring to that time period were significant between lactations (P<0.01). The assumption for such a result can be in the physiological response of the animal, ie the circular muscle (sphincter) on the top of the teat (*Mijić et al, 2005*).

Table 2. Relation between duration of tS500 (min) and LSCC according to lactations (LSM \pm SEM)

tS500 (min)	LSCC/Lactation							
	1.		2.		=>3.		Total	
	n	LSM	n	LSM	n	LSM	n	LSM
<= 0.10	30	2.84 \pm 0.31	30	2.93 \pm 0.30	38	3.07 \pm 0.24	98	2.96 \pm 0.16
0.11-0.20	66	2.70 \pm 0.19 ^a	32	3.31 \pm 0.26	76	3.26 \pm 0.20 ^b	174	3.06 \pm 0.13
0.21-0.30	37	2.73 \pm 0.27	25	2.46 \pm 0.43	37	2.97 \pm 0.27	99	2.75 \pm 0.18
0.31-0.40	15	1.89 \pm 0.43 ^A	14	2.40 \pm 0.44	24	2.68 \pm 0.35 ^B	53	2.38 \pm 0.23
>=0.41	9	3.77 \pm 0.51	12	3.25 \pm 0.43	12	3.17 \pm 0.38	33	3.37 \pm 0.25

Conclusion

Duration of tS500 is surely connected with the teat canal width and pre-stimulation phase. Quick or slow milking time (tS500) has negative effect on udder health, which was also confirmed in this research. For that reason, besides proper preparation of cows for milking, milking traits should be further improved through selection. Occurrence of bimodality of milk flow and unevenness in the amount of

milk among udder quarters open space for selection upgrade in breeding evaluation and selection of bulls' fathers and mothers.

Uticaj startnog protoka mleka na zdravlje vimena holštajn krava

Pero Mijić, Mirjana Baban, Maja Gregić, Franjo Poljak, Tina Bobić

Rezime

Protok mleka pri mehaničkom muženju krava sastoji se od više različitih faza koje mogu različito uticati ne samo na dužinu mužnje, već i na zdravlje vimena. Cilj istraživanja bio je da se procene efekti početnog protoka mleka (tS500) na zdravlje vimena. Istraživanje je sprovedeno na 457 krava holštajn rase. Zdravlje vimena (broj logaritamskih somatskih ćelija - LSCC) bio je najveći rizik kod krava koje su u trećoj laktaciji ($LSCC = 3,17 \pm 0,12$). Početni protok mleka (tS500) bio je jednak kroz sve laktacije, najduži kod krava u drugoj laktaciji ($0,21 \pm 0,01$ min). Najmanji efekat na zdravlje vimena ($LSCC = 2,38 \pm 0,23$) je kada je tS500 bio između 0,31 i 0,40 min. Duže i kraće trajanje početnog protoka mleka rezultiralo je značajno većim brojem somatskih ćelija u mleku krava holštajn rase.

Ključne reči: početni protok mleka, zdravlje vimena, holštajn krave

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FREQUENCY OF κ -CASEIN AND β -LACTOGLOBULIN GENOTYPES IN DAUGHTERS OF FIVE SIMMENTAL BULL SIRE

Dragan Nikšić¹, Vlada Pantelić¹, Dušica Ostojić Andrić¹, Predrag Perišić², Nenad Mičić¹, Marina Lazarević¹, Maja Petričević¹

¹ Institute for Animal Husbandry, Autoput 16, 11080, Belgrade-Zemun, Republic of Serbia

² University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080, Belgrade-Zemun, Republic of Serbia

Corresponding autor: Dragan Nikšić, draganniksic84@gmail.com

Invited paper

Abstract: The aim of this study was to determine the frequency of the κ -casein and β -lactoglobulin genotypes of the daughters of 5 Simmental bulls (sires) used for insemination in Serbia. Blood samples were taken from a total of 157 cows of the Simmental breed in Toplica and Rasina districts. Cows from which blood samples were taken were the daughters of the following bulls: Rorb B033, Dionis B52, Val B064, Rondi B029 and Res 1433. In all daughters of studied bulls, the presence of all three genotypes was determined, except for daughters of bull sire Res B1433, where the absence of genotype BB was determined. In addition to determining the frequency of genotypes and alleles for both milk protein fractions, based on the χ^2 test, a link between the sire and the presence of genotypes was determined, and in both cases a statistically significant relationship ($p \leq 0.001$) was found.

Key words: frequency, κ -casein, β -lactoglobulin, PCR-RFLP, bulls

Introduction

Milk proteins make up 3.0-3.5% of cow's milk and thus represent an important ingredient in feeding of their progeny and the necessary source of essential amino acids and biologically active proteins.

Protein synthesis is a process by which the cell builds proteins, for which the energy and the availability of nitrogen is required. *Ostojić (2007)* defines the milk protein synthesis as a process under control of hormone and

genetic mechanisms, which begins with transcription and ends with translation.

The polymorphism of β -lactoglobulin (β -Lg) and κ -casein (κ -CN) proteins is incorporated into modern cattle breeding programs, through which it aims to functionally improve populations. As the main protein of milk whey β -lactoglobulin is determined by the gene positioned on the 11th chromosome of cattle. *Caroli et al. (2009)*, with two dominant polymorphic forms (A and B), also indicate nine rare polymorphic variants (C, D, E, F, G, H, I, J, W). K-casein, as one of the four casein milk proteins, is determined by the gene positioned on the 6th cattle chromosome. *Caroli et al. (2009)* state fourteen polymorphic forms of κ -casein (A, A1, B, B2, C, D, E, F1, F2, G1, G2, H, I, J) of which two polymorphic forms dominate (A and B).

Genetic polymorphism of κ -casein and β -lactoglobulin in cows of different breeds in Croatia was studied by *Ivanković et al. (2011)*. Using the new analytical methods, they determined the share of the dominant allelic polymorphic variants of β -lactoglobulin and κ -casein in 3 commercial and 3 indigenous breeds of cattle: Holstein, Simmental, Brown cattle, Buša, Slavonian-Sremski Podolac and Istrian cattle. The share of allele B variant of β -lactoglobulin was dominant in all studied breeds of cattle (>52.9%). Allele A variant of κ -casein was dominant in selected breeds of cattle (60.7-76.4%), while the share of B variants of κ -casein was significantly more present in autochthonous breeds of cattle (48.2-84.1%).

Genetic polymorphism of fractions of milk proteins and their effect on the production properties of Simmental cows in Poland were studied by *Felenczak et al. (2006)*. These authors state that in the β -lactoglobulin a BD genotype was also identified with a frequency of 0.024. Other genotypes had the frequency: AA 0.236, AB 0.488 and BB 0.252, and alleles: A 0.486, B 0.502, D 0.012. For κ -casein three genotypes were found, AA, AB, BB, frequency 0.281, 0.498, and 0.221, respectively, while for alleles it was: A 0.530 and B 0.470.

In the study of the genetic polymorphism of κ -casein and its influence on the production performances of Simmental cattle, the autochthonous Busha breed, and the crosses of Simmental and Red Holstein cattle in Serbia, *Đedović et al. (2015)* report the following results: the genotypic incidence of κ -casein for Simmental breed were: 42.8; 47.6 and 9.6% for AA, AB and BB genotype, for crosses: 75.0; 25.0 and 0.0% and for Busha

animals: 41.7; 50.0 and 8.3%, respectively. The incidence of alleles A and B, for the observed breeds estimated on the basis of the genotype frequency, had values of 0.667 and 0.333 for Simmental breed, for crosses 0.875 and 0.125 and 0.667 and 0.333 for indigenous cattle breeds, respectively, while working with PCR-RFLP analysis of β -lactoglobulin of a small herd of Simmental cows in Serbia *Caro Petrović et al. (2017)* have found the existence of only AA β -lactoglobulin genotype.

Examination of the frequency of the κ -casein and β -lactoglobulin alleles in the Holstein-Friesian cows in Serbia was carried out by *Lukač et al. (2013)*. The study covered 765 Holstein-Friesian cows, daughters of 18 bull sires, for the determination of β -lactoglobulin genotypes and 420 cows for the determination of κ -casein genotypes. The following results were obtained: from 765 cows, 172 were AA, 448 were AB and 145 cows were BB β -lactoglobulin genotypes, which meant that the frequency of AA genotypes was 0.23, AB 0.58 and BB only 0.19. The frequency of alleles A and B was 0.52 and 0.48; of 420 cows, 105 were AA, 219 AB and 96 BB κ -casein genotypes. The frequency of genotypes AA, AB and BB ranged within values of 0.25, 0.52 and 0.23, while in alleles A and B, it was 0.51 and 0.49.

Material and Method

Blood samples for genetic testing were taken from 157 cows of Simmental breed in BD Vacutainer® K2EDTA tubes in an amount of 6 ml from the tail vein (v. Caudalis), after which they were stored at a temperature of 4°C until the DNA was isolated. The isolation of the DNA was performed using isolators according to the manufacturer's instructions. The multiplication of the portion of the κ -casein gene containing polymorphic sequence was done using the following primers (*Mitra et al., 1998*): Casein FW 5' CAC GTC ACC CAC ACC CAC ATT TATC - 3' and casein REV 5' TAA TTA GCC CAT TTC GCC TTC TCT GT - 3' (Invitrogen-Thermo Fisher Scientific Inc., USA). The multiplication of the portion of the β -lactoglobulin gene containing the polymorphic sequence was done using the following primers (*Medrano I Aguilar-Cordova, 1990*): β -lactoglobulin FW-GTC CTT GTG CTG GAC ACC GAC TAC A- 3' and

β -lactoglobulin REV-CAG GAC ACC GGC TCC CGG TAT ATG A- 3' (Invitrogen-Thermo Fisher Scientific Inc., USA).

The following steps were applied in the PCR reaction: denaturation at 95°C for 2 minutes, 30 cycles of denaturation at 95°C for 1 minute, 30 cycles of hybridization at 57°C (61°C for β -lactoglobulin) for 30 seconds and 30 cycles of polymerization at 72°C for 1 minute. The completion was followed by final elongation at 72°C for 7 minutes for κ -casein and 10 minutes for β -lactoglobulin. Identification of polymorphism in the genes for κ -casein and β -lactoglobulin was performed using a method based on restriction fragment size polymorphism (RFLP). The amplification products were purified by precipitation and treated with a restriction enzyme Hinf I that specifically recognizes the 5'GANTC-3' sequence, which includes polymorphism in the κ -casein gene and Hae III that specifically recognizes the 5'GGCC-3' sequence comprising polymorphism in the gene for β -lactoglobulin according to the manufacturer's instructions. Polymorphism of restriction fragment size was analyzed by agarose gel electrophoresis.

Statistical processing was performed in the SPSS Statistics for windows, Version 23.0 program, which included determining the frequency of genotypes for both investigated genes. By using the χ^2 test, the correlation between the sires and the genotype frequency was examined.

Results and Discussion

The research was carried out on 157 cows of Simmental breed on the territory of Toplica and Rasina district. In order to detect genotypes for κ -casein and β -lactoglobulin, specific primers were used. Polymorphism of restriction fragment size was analyzed by agarose gel electrophoresis, when three genotypes for both protein fractions were discovered.

The length of the restriction fragments of κ -casein for the AA genotype was 156, 132 and 91 base pairs, for AB 288, 156, 132 and 91 base pairs, while for the genotype BB the restriction fragment was 288 and 91 base pairs.

Table 1. Frequencies of κ -casein genotypes and alleles and the influence of bulls on the frequency

Sire, HB	Genotype	No. of animals	Frequency	Alleles	Frequency
Rorb,B033	AA	18	0.529		
	AB	14	0.412	A	0,735
	BB	2	0.059	B	0.265
Dionis,B52	AA	7	0.219		
	AB	19	0.593	A	0.516
	BB	6	0.188	B	0.484
Val, B064	AA	5	0.167		
	AB	15	0.500	A	0.417
	BB	10	0.333	B	0.583
Rondi, B029	AA	10	0.323		
	AB	16	0.516	A	0.581
	BB	5	0.161	B	0.419
Res, 1433	AA	13	0.433		
	AB	17	0.567	A	0.717
	BB	0	0.000	B	0.283
Total	AA	53	33.8		
	AB	81	51.6	A	0.596
	BB	23	14.6	B	0.404
$\chi^2=110.955$ df=8 p value=0.000					
χ^2 = hi square test; df= degrees of freedom; p = significance					

From Table 1 it can be seen that the presence of three genotypes for κ -casein with the frequency AA 0.529, AB 0.412 and BB 0.059 was found in daughters of Rorb HB V-033 bull sire, or by the number of cows AA 18, AB 14 and BB 2. The frequency of alleles for the daughters of this bull was 0.735 for allele A and 0.265 for allele B. The daughters of Dionis HB B52 had the following genotypes: AA 0.219, AB 0.593 and BB 0.188, while in 32 studied/tested daughters of this bull, the frequency of allele A was 0.516 and the frequency of allele B 0.484.

Genotypes AA, AB and BB were also found in daughters of bull sire Val HB V-064 with a frequency of 0.167, 0.5 and 0.333, respectively. In 30 daughters of this bull, the incidence of alleles A and B was 0.417 and 0.583, respectively and it was the only bull that was examined in this study whose daughters had a higher share of allele B compared to allele A for κ -casein. The same genotypes were also found in daughters of Rondi HB V-029 with frequencies of AA 0.323, AB 0.516 and BB 0.161. Allele A had a frequency of 0.581 while for Allele B it was 0.419. Daughters of bull sire Res HB 1433 are the only ones in this study in which BB genotype was not

represented. The frequency of the other two genotypes was 0.433 for AA and for AB 0.567. The frequency of alleles A in daughters of this bull was three times higher in relation to allele B and amounted to 0.717 compared to 0.283 allele B.

If we look at the frequencies of κ -casein genotypes on the total population, they are roughly the same as reported by *Dedović et al. (2015)*, *Falenczak et al. (2007)* and *Lukač et al. (2013)*, while *Ivanković et al. (2011)* obtained slightly different results of the frequency of genotypes.

Based on the χ^2 test of the independence of the trait, it was found that there was a statistically significant ($p \leq 0.001$) relation between the sire and the presence of genotypes for κ -casein.

The length of restriction fragments of β -lactoglobulin for the AA genotype was 144 and 108 base pairs, for AB 144, 108, 74 and 70 base pairs, while for the genotype BB the restriction fragment was 108, 74 and 70 base pairs.

In Table 2, it can be seen that in 34 daughters of Rorb HB V-033, three genotypes for β -lactoglobulin with a frequency of AA 0.371. AB 0.486 and BB 0.143 were found. The allele frequency was 0.632 and 0.368. Bull sire Dionis HB B52 had 32 examined daughters with a genotype frequency of 0.313 AA, 0.406 AB and 0.281 BB. Allele A had a frequency of 0.515 and allele B 0.484.

As with the previous two bulls, in daughters of bull sire Val, HB V-064, the highest frequency was recorded for AB genotype of 0.5, slightly lower for BB 0.4 while AA genotype had the lowest frequency of 0.1. The allelic frequency ranged from 0.35 for allele A to 0.65 for allele B. The frequency of the genotypes was tested in 31 daughters of the bull Rondi HB V-029. The AA genotype had a frequency of 0.387, somewhat higher frequency was record for genotype AB. 0.548, while the BB genotype had the lowest frequency of 0.065. Alleles A and B. expressed the distinctive difference in the frequency with values of 0.661 and 0.339.

As with κ -casein, in case of β -lactoglobulin, the BB genotype has not been identified in 30 daughters of the bull Res HB 1433, and of the remaining two genotypes the higher frequency was recorded for AB genotype of 0.533 while the AA genotype had a frequency of 0.467. The absence of the BB genotype for κ -casein and β -lactoglobulin in daughters of the bull Res HB 1433 leads to the conclusion that this bull is an AA

genotype, which is very important if we know that the genotypes of these protein fractions affect the production and the composition of the milk.

If we look at the frequency of genotypes for β -lactoglobulin on the total population, they are about the same as reported by *Lukač et al. (2013)* in their research, while *Ivanković et al. (2011)*, *Caro Petrović et al. (2017)* and *Falenczak et al. (2007)* show somewhat different results of the frequency of genotypes.

Based on the χ^2 test of the independence of the trait, it was found that there was statistically significant ($p \leq 0.001$) relation between bull sire and the presence of β -lactoglobulin genotypes.

Table 2. Frequencies of β -lactoglobulin genotypes and alleles and the influence of bulls on the frequency

Sire HB	Genotype	No. of animals	Frequency	Alleles	Frequency
Rorb. B033	AA	13	0.371	A B	0.632 0.368
	AB	17	0.486		
	BB	4	0.143		
Dionis.B52	AA	10	0.313	A B	0.516 0.484
	AB	13	0.406		
	BB	9	0.281		
Val. B064	AA	3	0.100	A B	0.350 0.650
	AB	15	0.500		
	BB	12	0.400		
Rondi. B029	AA	12	0.387	A B	0.661 0.339
	AB	17	0.548		
	BB	2	0.065		
Res. 1433	AA	14	0.467	A B	0.733 0.267
	AB	16	0.533		
	BB	0	0.000		
Total	AA	52	32.48	A B	0.580 0.420
	AB	78	50.32		
	BB	27	17.20		
$\chi^2=129.074$ df=8 p value=0.000					
χ^2 = hi square test; df = degree of freedom; p = significancet					

Conclusion

Determination of the κ -casein and β -lactoglobulin genotypes in bulls used for insemination of cows of specific population is very important because by monitoring various forms of milk proteins and their frequencies

we can increase the frequency of those genotypes that exhibit a beneficial effect on the milk properties and fulfill the goals that a breeder has for the particular populations of cows. By favoring suitable genotypes, i.e. by the planned selection of parents of desirable genotypes, first of all sires (bulls), a faster genetic improvement of the population is realized, as well as improvements in both production and milk composition and reduction in production losses.

Frekvencija genotipova κ -kazeina i β -laktoglobulina kod ćerki pet bikova simentalске rase goveda

Dragan Nikšić, Vlada Pantelić, Dušica Ostoji Andrić, Predrag Perišić, Nenad Mičić, Marina Lazarević, Maja Petričević

Rezime

Cilj ovog istraživanja bio je da se utvrdi frekvencija genotipova κ -kazeina i β -laktoglobulina ćerki 5 bikova (očeva) simentalске rase koji se koriste za osemenjavanje u Srbiji. Uzorci krvi uzeti su iz ukupno 157 krava simentalске rase u Topličkom i Rasinskom okrugu. Krave od kojih su uzeti uzorci krvi bile su ćerke sledećih bikova: Rorb B033, Dionis B52, Val B064, Rondi B029 i Res 1433. Kod svih ćerki, ispitivanih bikova, utvrđeno je prisustvo sva tri genotipa, osim kod ćerki bika Res B1433, kod kojih je utvrđeno odsustvo genotipa BB. Pored određivanja frekvencija genotipova i alela za obe frakcije proteina mleka, na osnovu χ^2 testa urađeno je ispitivanje veze između očeva i zastupljenosti genotipova, i u oba slučaja je utvrđeno da postoji statistički značajna veza ($p \leq 0,001$).

Ključne reči: frekvencija, κ -kazein, β -laktoglobulin, PCR-RFLP, bikovi

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SCIENTIFIC DIRECTIONS OF SHEEP BREEDING DEVELOPMENT IN RUSSIA

Marina I. Selionova¹, Magomet M. Aybazov¹, Milan P. Petrovic², Galina T. Bobryshova¹, Violeta Caro Petrovic²

¹All-Russian Research Institute of Sheep and Goat Breeding – branch of the North Caucasus Federal Agricultural Research Center, Russia

²Institute for Animal Husbandry, Belgrade-Zemun, Serbia

Corresponding author: Marina I. Selionova, m_selin@mail.ru

Invited paper

Abstract: The article analyzes the state and prospects of scientific support for sheep breeding in Russia. The data on the production and export of wool are given. It is shown that against the background of decrease in a demand for natural fibers (wool), there is an increase in mutton production volumes in the world and in Russia, which determines the development of industrial technologies for the production and deep processing of mutton as priority areas for scientific research.

The data are presented in favor of the fact that the prospects for the production of lamb meat and mutton in Russia should be considered simultaneously in two directions: the first is meeting the needs of the domestic market and the second is the possibility of large export deliveries. The latter is promising and is dictated by the more favorable geographical position of Russia, compared with leaders of world mutton export, Australia and New Zealand, to the countries of the Middle East and Western Europe, where the use of mutton is traditional.

Key words: sheep breeding, the priorities of scientific support

Introduction

Sheep production in the world is moving forward with dynamic steps thanks to the development of science. The need for sheep products is growing which also improves sheep production (*Petrovic et.al. (2013); Selionova (2018)*). According to many authors, a number of factors influence the effect of sheep production (*Kholmanov, 2015; Ulyanov and Kulikova, 2017; Erokhin et al., 2017*).

Accelerated development of the agro-industrial complex in Russia is among the priority state programs. At the present stage, the task is set not only to increase the volumes of agricultural products in order to ensure the food security of the country, but also to increase its quality and competitiveness.

Sheep breeding, as a unique branch capable to produce products using efficiently extensive natural forage resources, including desert and semi-desert, mountain and high-mountain pastures, inaccessible to other branches of animal husbandry, is assigned a definite role in solving the problems posed.

At the same time, determining the vector of Russian sheep breeding modern development, one should proceed from the general tendencies in the state and development of the branch in the world, the demand and price conjuncture for sheep products, its consumer properties.

In this regard, there are several analytical calculations about the state of world sheep breeding and the role of our country in it.

The aim of this review paper is to give a scientific overview of the future development of Russia's sheep breeding.

Trends of sheep production in the world

The number of sheep in the world for 50 years ranges from 1.0 to 1.2 billion. Previously, the USSR, Australia, China, New Zealand and India dominated on this indicator. Since the beginning of two thousands, China has been leading with a big advantage (202 million) (Figures 1 a, b).

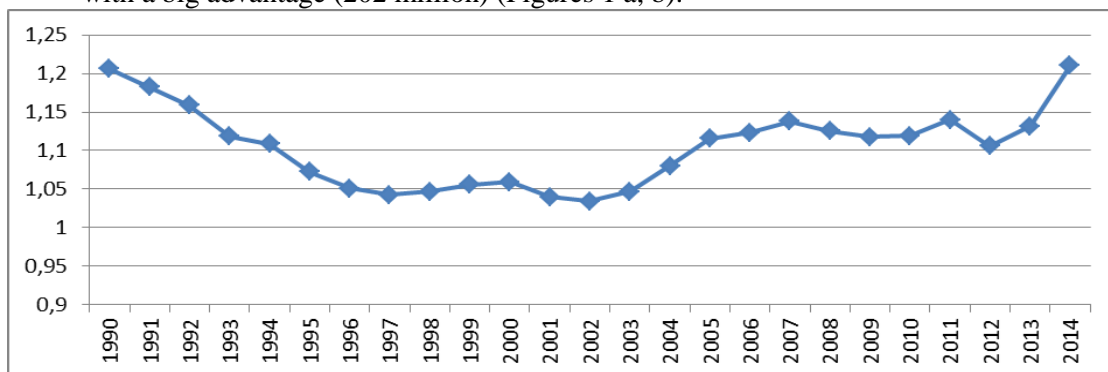


Figure 1 a: The number of sheep in the world, billion

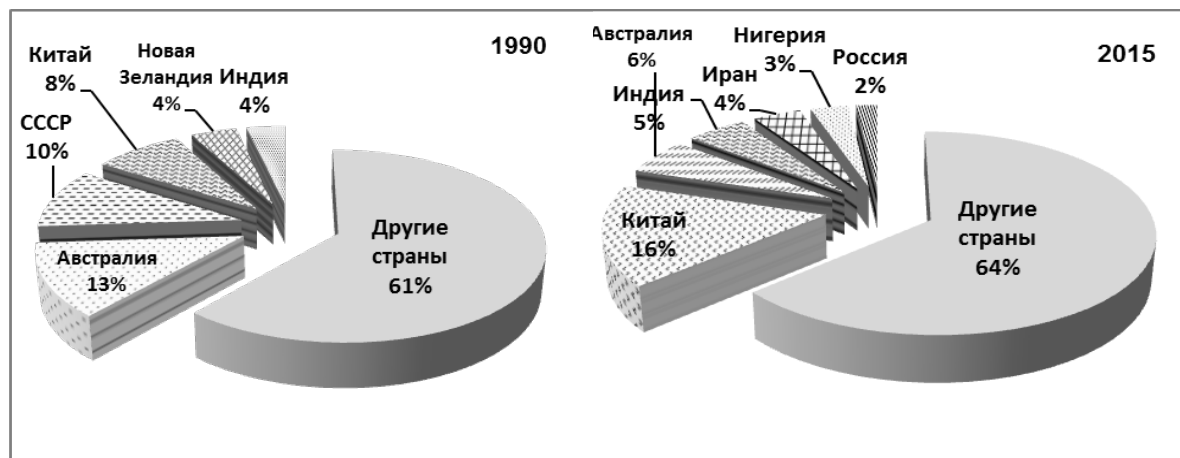


Figure 1 b: The proportion of the sheep number in the leading countries of the world

World wool production has been steadily decreasing since 1990, over past 25 years, the decline has made 36.5% (Figures 2), while the production of mutton steadily increased: an increase over the specified period was 70% (8.6 million tons).

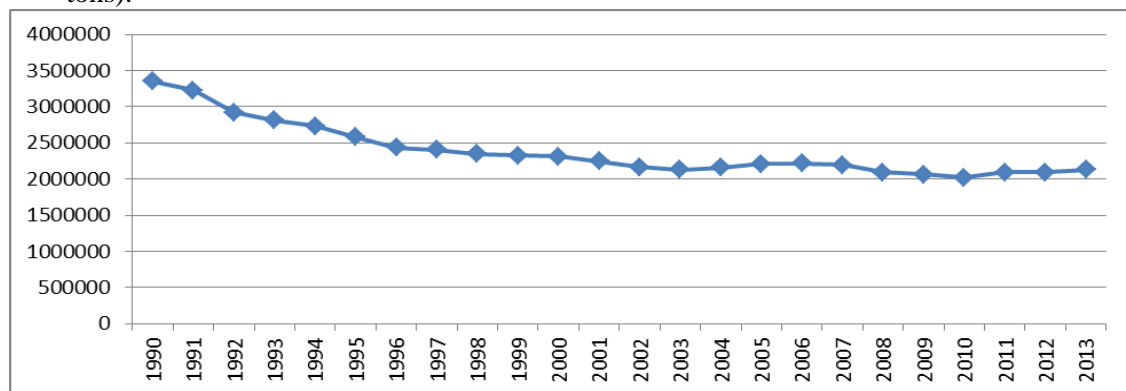


Figure 2. World wool production, thousands of tons

Since 2000, China has been leading in wool and mutton production, at the same time, in wool production its superiority over the second large producer (Australia) currently stands at 31%, and in the mutton production its advantage is almost 3 times. A comparison of the data presented above indicates that in the past twenty-five years, global sheep breeding has been reorganized from wool to meat (Figures 3 a, b).

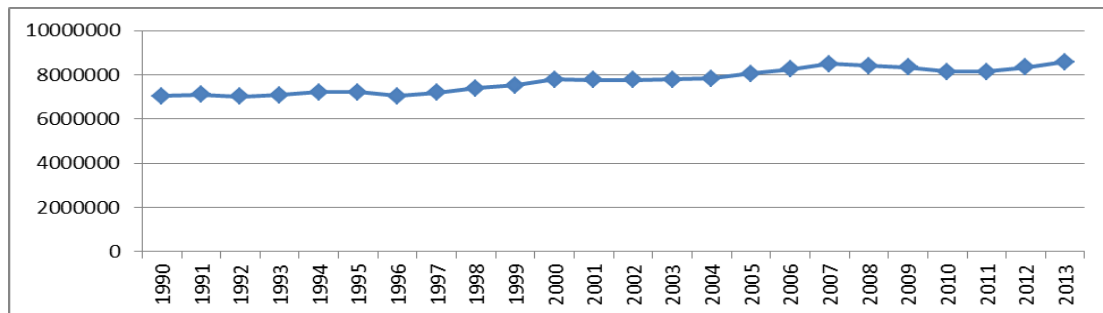


Figure 3 a: Mutton production in the world, million tons

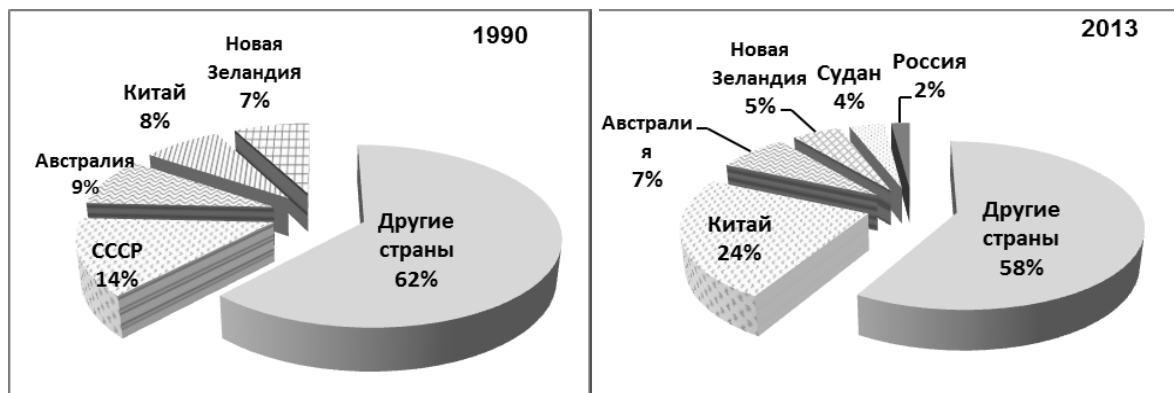


Figure 3 b: The share of mutton production in the leading countries of the world

Trends of sheep production in the Russia

Similar processes, that is, a decrease in the share of purebred merino sheep breeding (over 10 years by almost 25%) and an increase in semi-coarse, coarse-hair and crossbred sheep stock for the production of mutton, occurs in sheep breeding of Russia (*Dunin et al., 2018*)

The reduction of sheep wool direction was due to a significant decrease in demand for wool due to the rapid development of the chemical industry and increasing production volumes of synthetic fibers. Textile and consumer properties of artificial fibers are improving from year to year, and we should not expect an increase in demand for natural fibers, including wool, in the near future. Nevertheless, as mentioned above, the number of sheep in the world in 1990-2015

was stable, and this testifies that in the world in recent times sheep have been bred to a greater extent for meat production. And this circumstance should be considered as the main challenge of our time for the scientific substantiation of the development directions in Russian sheep breeding in the near future.

Russia, until 1990, was a country of highly developed sheep breeding; first of all it concerns fine-wool sheep breeding (*Erohin et al., 2014*). Sheep breeding was a strategic industry, fulfilling first of all the state order that is increasing the combat capability of the army through the supply of quality uniforms for military personnel. Another guaranteed consumer of sheep breeding products in Russia was the education system, which provided for obligatory manufacturing of school uniforms from woolen and half-woolen fabrics. All the links of the powerful wool-manufacturing state-consumer complex were adjusted in such a way that sheep breeding was an efficient industry and organically fit into the Russian economy of the pre-reform period. In 1985, manufacture of woolen fabrics reached 824 square meters, which allowed Russia to take a leading position.

Scientific researchers also met the needs of the time. Russian sheep breeding science has rightfully received worldwide recognition. However, with the intensive development of the chemical industry, the creation of a large assortment of modern synthetic fabrics, some of them were closer to natural fibers in consumer qualities, and exceeded certain parameters, while competing in price and manufacturability, the value of wool as a strategic raw material is not considered now any more. The adoption of a new military uniform, a uniform for schoolchildren led to the absence of a guaranteed state order, which radically changed the situation in the industry. The period of the market transformation is the slow destruction of the world's largest wool complex in Russia.

An alternative system, in which all strata of the population would be consumers of domestic high-quality woolen products, has not yet been created. Due to technological backwardness, the remaining domestic textile enterprises cannot produce fabrics that are competitive in the global market. Due to the low profitability of textile enterprises and the lack of sufficient funds, they are not able to offer the manufacturer the appropriate price for high-quality raw materials, namely, fine merino wool up to 23 microns. In this regard, the best high-quality wool, which is produced mainly in the North Caucasus region, and in particular in the Stavropol region, is exported abroad. Thus, according to IWTO data, the export of Russian wool in physical weight in 2015 amounted to 12.1 thousand tons of greasy wool and 7.5 thousand tons of washed wool.

Taking into account the current situation, the state, along with such types of support as reimbursement for the cost of breeding stock keeping and breeding work, in 2015 introduces a new form, that is, subsidies for the production and sale of fine and semi-fine wool in the amount of 153 million rubles. The total volume of

the federal budget funds provided for in the up-dated State Program for the Development of Agriculture by 2017 amounted to about 900 million rubles. Thus, state support, on the one hand, will stimulate the production of high-quality fine and semi-fine wool in large breeding farms, and on the other hand, will help provide domestic enterprises of the textile and light industry with high-quality woolen raw materials.

Currently, 24.2 million sheep are bred in Russia, with the main sheep stock being kept in peasant farms and private farms. In different regions, this proportion ranges from 60 to 80%. However, due to uncontrolled crossing, sometimes the absence of elements for zoo technical accounting, and, accordingly, breeding work does not allow to consider this sector as a supplier of raw materials with the required quality for the processing industry. However, the outlined deficit of wool, which is further aggravated by the unprofitability of imports, in the near future will inevitably raise the question of involving peasant and private farms in the forming wool complex, which provides for a well-built streamlined system from the manufacturer to the wool worker. Of course, this process will be carried out through mutually beneficial cooperation of all interested parties. At the stage of reorientation of small producers to the production of wool demanded by textile enterprises, an effective measure can be reimbursement by the state of costs (up to 50%) for the purchase of pedigree pure bred animals.

Stimulating of wool production and its processing into the final competitive product will contribute to the creation of high-tech jobs, employment of the population and, ultimately, an increase in production volumes. A positive example is the new innovative enterprise “Quest A”, which uses a unique technology for wool scouring and washing, which has no analogues in the world involving the repeated use of the mineral water from underground sources.

Against the background of objective difficulties in the production, sale and processing of wool, promising for merino sheep breeding can be the development of economically advantageous genotypes and their distribution in region, where sheep breeding is profitable and there is no alternative agrarian production [1, 3].

This is the way the sheep breeders of Australia, South Africa have gone. More than thirty years ago, work began in these countries on the creation of merino sheep characterized by high meat productivity, fine wool (21-22 microns) and fertility. New breeds were bred, such as the South African meat merino, done merino, afrino. The positive experience of foreign colleagues largely determined the advisability and need for work on the development of the domestic breed in a similar direction. In 2004, with a separate experiment, and since 2007 on a large scale, work began on the use of done merino gene pool to get a solid mass of animals in the type of meat merino. In 2015, a sheep population of more than 5,000 breeding stock was got with significant differences for reasons of expressiveness in

meat production, wool fiber fineness and fecundity. Animals of the new genotype differed evenness in the indicated characteristics, which were stable transmitted to the offspring. Further work in the domestic fine-wool sheep breeding, both in the classic wool and in the new meat and wool, will be aimed at the optimal combination of meat, wool productivity and the demands of the consumer market [5].

Scientific view of the future sheep farming

The undoubted priority of the next decade will be the development of production technology and deep processing of mutton of an industrial basis [5]. If in respect of one type among the products of sheep breeding (wool) there were quite clear prospects, then there is no definite answer in relation to the other important economic component of the industry – mutton.

In Russia, unfortunately, there has never been an organized mutton market in the broad sense of this term. The desire of producers to independently enter both the internal and external markets was not crowned with success. And the lack of government interest and support in this segment also adversely affected domestic sheep breeding and the processing industry. Involvement of processing enterprises into the active process, protection of the economic interests of Russian producers can be effectively implemented only under the condition of state protectionism.

For example, in Europe, the United States, mutton production is subsidized by 30-45%, wool by 10-15%, and crop production even more – up to 70%. That is, the impressive state support of the own agricultural producer is obvious.

It is known that, both abroad and in our country, carcasses not exceeding 16-18 kg, obtained from animals not older than 7-8 months, are in demand in the market. In this case, the meat should have the appropriate parameters: color, density, flavor, a certain amount of fat inclusions, that is, the entire set of indices characterizing its high consumer qualities. One of the reserves for satisfying of such a request and getting animals with a high rate of increasing the muscular weight can be purposeful commercial crossing. Numerous studies have shown that when commercial crossing of fine-wool ewes with rams of meat breeds, the average daily gains of live weight, carcass yield, meatness ratio, quality characteristics of mutton increase significantly, while reducing feed costs by 1.0-1.5 fodder units when obtaining one kilogram of weight gain. In addition, the fecundity of females, the survival rate of offspring, meat production per one ewe increase, which ensures the profitability of obtaining young mutton.

Another significant reserve for obtaining high-quality products is coarse-woolen sheep breeding, whose share in Russian sheep breeding over the past 10 years has increased more than 4 times. Of course, the presence of a significant

amount of natural forage lands plays an important role in this process: highly productive alpine and subalpine pastures in the republics of Dagestan, Karachay-Cherkessia, and Kabardino-Balkaria. According to the yield of herbage, the content of digestible protein in it, the fodder units, mountain and pre-mountain pastures are 1.5-2.5 times higher than plain pastures, and when compared with the degraded steppe pastures of the eastern part of the Stavropol region, certain districts of the Republic of Kalmykia, Rostov and Astrakhan regions, the difference will be even more significant.

With any approach, the production of young mutton, lamb meat requires new technological solutions, which include resource-saving technology. In addition, a wise approach to the use of previously developed technologies should be made. Individual elements of industrial technology should be adapted to modern conditions, in particular, large fattening grounds for sheep young. At the same time, it is important to combine them with modern slaughterhouses and, most importantly, consumer cutting and packaging of mutton (*Erokhin et al. 2017; Amerkhanov et al., 2018*).

Considering the aspects constraining the development of the domestic mutton market, it is impossible not to note the biological characteristics of sheep reproduction, a pronounced seasonal nature of reproduction. This does not allow to get young animals for fattening during the year, which is why it is almost impossible to have invariable guaranteed supply of mutton to retail chains. While along with the quality requirements of young mutton, the cyclical nature of supply is the main condition of large supermarkets.

One approach to solving this problem could be the creation of a large agricultural holding with a closed cycle: from production to modern cutting and packaging.

In this direction, large investments and government support are needed. An example of this approach is the 'Miratorg' company, which intends to invest about 20 billion rubles in the production of mutton on industrial basis.

Interesting in this direction is the experience in the work of the largest Australian company "Thomas Foods" in the deep processing of mutton, where 11 thousand sheep are slaughtered daily. Products are shipped to 25 countries. At the carcass cutting stage, lots are formed taking into account the preferences and purchasing power of consumer countries. Stable operation of this company provides about 60% of the sheep breeding economy in South Australia. The annual turnover exceeds 1 billion dollars.

Here it is necessary to emphasize that the prospects for the production of lamb meat and young mutton in Russia should be considered simultaneously in two directions: the first is meeting the needs of the domestic market and the second is reaching large export deliveries. The latter is promising and dictated by the more

favorable position of Russia, compared with the leaders of world mutton exports (Australia and New Zealand), to the countries of the Middle East and Western Europe, where the use of mutton is traditional.

Conclusions

The main vectors of sheep breeding development in Russia and its scientific support in the near future, apparently, will be as follows:

- working out of the strategy and tactics of development in individual areas of the branch, including the scientific rationale for priority;
- the economic substantiation of the sheep products parameters produced in various environmental climatic and economic conditions;
- formation of an organized market for sheep products;
- formation of marketing technologies for the popularization and promotion of sheep breeding products.

In this direction, it is necessary in the shortest possible time to develop new resource-saving, low-cost and environmentally friendly technologies in keeping sheep for large producers, as well as small forms for entrepreneurship, new designs of machines, mechanisms and equipment, optimize the system for feeding and keeping young animals in the production of lamb meat, young mutton, including from sheep of specialized meat breeds.

Naučni pravci razvoja ovčarstva u Rusiji

Marina I. Selionova, Magomet M. Aybazov, Milan P. Petrovic, Galina T. Bobryshova, Violeta Caro Petrovic

Rezime

U radu se analiziraju stanje i perspektive naučne podrške ovčarstvu u Rusiji. Navedeni su podaci o proizvodnji i izvozu vune. Pokazano je da na pozadini smanjenja potražnje za prirodnim vlaknima (vunom) dolazi do povećanja količine proizvodnje ovčetine u svetu i Rusiji, što određuje razvoj industrijskih tehnologija za proizvodnju i dubinsku obradu ovčetine kao prioriteta područja za naučno istraživanje. Podaci predstavljeni u radu govore u prilog činjenici da perspektive za proizvodnju jagnječeg mesa i ovčetine u Rusiji treba razmatrati istovremeno u dva pravca: prvi je zadovoljenje potreba domaćeg tržišta, a drugi mogućnost velike izvozne mogućnosti. Ovo poslednje obećava i diktira povoljniji geografski položaj

Rusije, u poređenju sa liderima svetskog izvoza ovčetine, Australije i Novog Zelanda, u zemlje Bliskog Istoka i Zapadne Evrope, gde je upotreba ovčetine tradicionalna.

Ključne reči: uzgoj ovaca, prioriteti naučne podrške

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CONDITION AND PROSPECTS OF SHEEP BREEDING DEVELOPMENT IN KAZAKHSTAN

Yessenbay I. Islamov¹, Gulzhan A. Kulmanova²

¹Non-commercial JSC "National Agrarian Science and Educational Centre", Kazakhstan

²Kazakh National Agrarian University, Kazakhstan

Corresponding author: islamov_esenbay@mail.ru; gulzhan_62@mail.ru

Invited paper

Abstract: The article deals with the state and prospects of development of sheep in Kazakhstan. In article questions of a present state and further development of breeding sheep breeding in Kazakhstan are considered. In the concept of entering the Republic of Kazakhstan among the 30 most developed countries in the world, it is necessary to solve a number of problems in the most promising areas, one of which is the transfer to the innovative rails of the agricultural sector of the country, by introducing highly efficient technologies and working on the basis of the best international standards. Under these conditions, the leading role in solving the problem is played by the intensive development of sheep breeding, which provides the population with the need for specific types of raw materials and food. Sheep breeding is an important branch of animal husbandry. Unlike many other farm animals sheep give the largest quantity of various products: wool for various purposes, mutton fat raw, milk, sheepskin and fur-raw hides, Saski. The list of products produced from sheep products is even more extensive: fabrics and knitwear, felt and felted, fur, fur and leather products, numerous food products. Such a variety of products and products from it is provided by a large number of breeds of sheep.

Key words: breed, the gene pool of the herd, line, selection and breeding

Introduction

Prior to the socialist reconstruction of agriculture, sheep breeding in Kazakhstan was represented almost exclusively by rough-haired sheep. In 1928-1929 the specific weight of fine-wool livestock and fine-woolly-coarse-wool hybrids was less than 1% to the total number of sheep of the Republic. Local (Kazakh) sheep accounted for about 95% of the population, the rest of the livestock was represented by animals of other coarse-haired breeds: volosh, Mikhnov, Russian long-tailed-scrawny, brought to Kazakhstan by immigrants from the

European part of tsarist Russia in the pre-revolutionary period. A small number of Karakul sheep were bred in some areas of South Kazakhstan (now Turkestan) region. In Kazakhstan during that period (as well as before revolution) were engaged, in essence, only meat-greasy sheep breeding. On the basis of socialist reconstruction of agriculture it became possible to implement the necessary measures to improve the quality of sheep.

The development and growth of the economy of the Republic of Kazakhstan, welfare depends on the rise of agriculture and animal husbandry. In our country, namely in the Republic of Kazakhstan, there is a great potential for the development of livestock and agriculture, due to the presence of natural conditions and historical experience.

Historically, Kazakhstan was and is the largest producer of livestock products. The products of cattle breeding, sheep breeding, pig breeding, poultry farming and other industries produced earlier in our country were enough to fully provide the population not only of the Republic, but also of other countries of the near and far abroad. It is known that as a result of the current economic reforms from 1985 to 1995, the livestock of Kazakhstan suffered huge losses, both quantitatively and qualitatively, where a special place is occupied by the loss of livestock breeding in all sectors of livestock (*Kulmanova, 2005*)

Quantity of sheep

According to FAO (2010), the number of sheep in the world has more than 2,300 breeds of sheep, which are characterized by a wide range of its specialization: wool, fur, meat, meat-fat, dairy, coarse and dry, etc. The value of sheep in the national economy is not limited only to the products. According to FAO, in 2017 the number of sheep in the world was 1202.4 million heads, and in 1990 it was 1205.6 million heads. During the period 1990-2017 the number of sheep in the world decreased by 0.3 %, almost unchanged. In the CIS countries (FAO data) in 2017 was 89.4 million. goats, and in 1991 – 140,2 million goats. During the period 1991-2017 the number of sheep in the CIS countries decreased by 36.3 %. The reasons for the sharp decline in the number of sheep in the CIS countries have a similar origin – a change of ownership; lack of demand for the domestic market of Merino wool; mismatch of purchase prices for the products of the industry and the cost of its production; disparity of prices for industrial and agricultural products; intervention of the domestic market with cheap imported goods from wool, cotton, leather; not preparedness and protection of domestic producers from the elements of the market by the state (*Statistical review of Kazakhstan, 2018*).

Lamb production

For the modern stage, it is characteristic that the central link in sheep breeding has been transferred from wool productivity to meat and dairy. This reassessment of the industry's products is associated with an acute shortage of food for the population, especially in relation to animal protein, the main sources of which are meat, milk, eggs, and fish. As a result, the currently economically significant sheep products are meat and milk, whose share in the gross income from the sale of all products received from these animals is 85-90% or more. According to the FAO for the period 1990-2017, with almost the same number of sheep, lamb production in the world increased by 35%, from 7033.5 thousand tons in 1990 to 9498.3 thousand tons in 2017. This indicates that the issues of increasing the meat productivity of sheep in the world are great attention. In the CIS countries for the period 1991-2017 while reducing the number of sheep by 36.2%, mutton production increased by 3.8%, from 906.0 thousand tons in 1991 to 940.9 thousand tons in 2017. It should be noted that 3.8% is average for the CIS countries, but the pace of increase in mutton production in different CIS countries was different. Thus, the level of the landmark 1991 in 2017 was reached or exceeded in Uzbekistan (369%), in Turkmenistan (358%), in Tajikistan (270%), in Azerbaijan (240%), in Armenia (122%), and in other CIS countries this figure was: in Ukraine - 19%, in Moldova - 55%, in Kazakhstan - 56%, in Russia - 58.5%, in Kyrgyzstan - 90%. In recent years, meat production (kg/year) per capita in the world was 42.7 kg, including: poultry meat - 14.9; pork - 15.5; beef - 9.3; lamb - 1.2; other meat - 1.8 kg. In Russia, these figures are as follows: pork - 17.9; poultry meat - 23.4; beef - 11.5; lamb - 1.2; goat meat - 0.1; other meat - 2.7; meat of all types - 56.8 kg. Among the CIS countries, relatively high rates of production of meat of all types per capita are Belarus (112.9 kg), Turkmenistan (60.1 kg) and Kazakhstan (57.4 kg.), the lowest - Georgia (9.9 kg) and Tajikistan (10.6 kg), and Turkmenistan (25.7 kg), Azerbaijan (8.2 kg), Kazakhstan and Kyrgyzstan - 7.8 kg each lead in the production of mutton per capita (*Statistical review of Kazakhstan, 2018*).

Wool production

FAO filed the production of wool of all kinds in the world for the period 1980-2016, with a stable sheephead, it decreased by 25%, from 2,794.2 thousand tons in 1980 to 2099.2 thousand tons in 2016. In Kazakhstan, wool production decreased from 105.0 thousand tons in 1990 to 22.9 thousand t in 2000, and 34.6 thousand tons in 2017.

Sheep farming is a traditional livestock industry in Kazakhstan. Kazakhstan sheep breeding is represented by more than 20 breeds, plant, intrabreed types and various offspring of breeds and is not only one of the leading livestock industries, but also has great potential, which is facilitated by the presence of large areas of natural pastures in the Republic of Kazakhstan - more than 187 million hectares. In Kazakhstan, large grazing areas are located in desert and semi-desert lands. Effective use of these pastures, as shown by many years of practice, is only possible by breeding sheep. No other type of farm animal is more efficient than sheep in using such low-yielding lands. The change of ownership in agriculture led to a sharp reduction in the number of sheep and a decrease in their productivity (*Kulmanova, 2005*). So, if at the beginning of 1992, there were 34.5 million Ovecians and goats in all categories of Kazakhstan's farms, then, in 2018, only 18.5 million. The breeding base of sheep breeding has also changed. So, if at the beginning of 1990 there were 241 breeding farms, in which there were 18.3 million breeding sheep, then on January 1, 2018 the proportion of breeding sheep in the total livestock is only -5.5%. The current state of the industry sheep breeding requires a scientifically based program for the future, defining both the basic concepts of its development and measures of state support for the industry. In this regard, the primary task should be the preservation of the valuable sheep gene pool - the basis for the further development of sheep breeding in Kazakhstan. The priority areas of scientific support for sheep breeding are: preserving and improving the existing breed gene pool of animals, including rare, small, endangered breeds and offspring; , types, lines and competitive breeding herds; development of effective methods of herd reproduction biotechnology; improvement of sod technology neighing, feeding, producing and processing sheep products.

Industry presented 5 areas of productivity with identified breed sheep resources: fine-wool, semi-fine-wool, semi-coarse, coarse and dry. Meat-greasy sheep. One of the priorities of the modern development of sheep breeding in Kazakhstan is meat and greasy sheep breeding, which includes semi-coarse and coarse-haired directions and is of great importance in meeting the needs of the population in meat and meat products. The development of meat and greasy sheep breeding is facilitated by the vast pastures of the republic, which are most rationally used by the fat-tailed and Karakul sheep. Meat-fat sheep breeding in Kazakhstan is a traditionally established branch of animal husbandry. It should be noted that since ancient times, the Kazakhs, with other things being equal, give preference to the breeding of fat-tailed sheep, receiving from them not only high-calorie dietary meat with high taste, but also fat-tailed fat used in the treatment of certain diseases. In the main breeding zone, meat-tallow sheep. In the future, it is necessary to strengthen the development and implementation of breeding

techniques for improving the breeding and productive qualities of the Edilbayev, Saryarka, Ordabinsk and Kazakh fat-tailed semi-coarse wool and improved Kazakh fat-tailed coarse-haired sheep in order to bring the production of meat to 1 ewe up to 38-40 kg. Along with the increase in the productive qualities of the existing breeds of meat and greasy sheep, the creation of new types of Kazakh fat-tailed sheep with coarse lightened wool is envisaged as well as the preservation and reproduction of rare and endangered types and offspring of Kazakh fat-tailed coarse-haired sheep. In the flocks of the Kazakh fat-tailed semi-coarse-haired and Saryarka breeds, work will be continued to consolidate the animals of the desired type, in its morphological composition of the desired types of fibers. At the same time, the possibility of cross-breeding of Kazakh coarse-haired queens with Kargaly sheep of the Kazakh fat-tailed semi-coarse-haired breed is not excluded. It will also be effective to use separate offspring of coarse-haired sheep, distinguished by white color of wool and rather high meat and greasy qualities. Kurdish sheep are competitive in meat production relative to sheep of other productivity areas due to high slaughter and meat-greasy qualities and most importantly - low cost of mutton production account of the feeding of animals on natural pastures. Thin-sheep sheep breeding. In Kazakhstan, the origin of fine-wool sheep breeds: Kazakh fine-fleeced, arharomerinos, South-Kazakh Merino, North-Kazakh and meat-Merino. New breeds must have good adaptability to grazing conditions and at the same time produce enough meat and wool of high quality, be competitive and be in demand in the market. Therefore, the breeds were created only for certain breeding zones, for example, the Kazakh fine-wool and meat-merino for large areas of South-Eastern Kazakhstan, the South Kazakh and Kazakhstan merinoes for the territories of the south of the republic, the North Kazakh merinoes, mainly for the north and north-east, the Kazakh arharomerynes for based on the external and domestic market conjuncture, the competitiveness of the domestic fine wool and the profitability of its production should be ensured through productivity of breeds of sheep in the direction of creation: pedigree herds with fineness wool of 60-64 quality and trimmed scoured wool in females - 3.0-4.0 kg, live weight 60-65 kg, fertility 130-150% based on pure breeding with using rams from leading breeding farms, as well as partial introductory crossing with Australian merino. An important link in breeding work in this direction is the preservation of large size, higher precocity and adaptation of sheep to local conditions, typical of domestic fine-fleeced breeds; A new fine-wool breed "Kazakhstan merino" was created in 2015 on the basis of a reproductive cross-breeding of ewes, the South Kazakh Merino (SKM) with rams of the best Australian breeds, which produces merino wool at the level of world standards. Promising areas for increasing the production of lamb from fine-wool sheep, along with the technology of preparing them for slaughter, is the improvement of their reproductive and meat qualities, including

the use of interbreeding. To this end, in 2011 in Kazakhstan, a fine-wool breed of sheep of meat type (etti-merino) was bred using the purebred breeding and crossing of Kazakh fine-wool queens with German Merinofleischschaff sheep, imported from Germany. As a result of a long-term purposeful breeding work of a team of scientists and specialists of the economy in the Almaty region, a highly productive flock of Kazakh fine-fleeced sheep - "Zharkentsky" interbreed type, whose productivity exceeds the breed standard for live weight and hair wool by 15 and 20% is created. A distinctive feature of sheep of this type is: high adaptive properties, polyesterism, large live weight, large size and production of merino wool 64-70 quality with high technological properties. Analysis of the research and production work of domestic and foreign scientists shows that half-fine meat and wool sheep breeding acquires one of the promising areas in animal husbandry (*Kulmanova et al., 2014*). The development of early meat and wool sheep breeding is due to their dual productivity, early maturity, high slaughter qualities and fecundity. Therefore, the development of modern meat and wool sheep, compared with other areas, should be characterized by the highest rates of fecundity, weight gain and slaughter qualities, which characterizes this direction of sheep breeding, as the most cost-effective and intensive. This will make it possible to supply competitive young lamb and crossbred wool to both the domestic and foreign markets. It should be noted that lambs, aged 4-4.5 months, weigh 30-32 kg, and give excellent carcass 15-16 kg, highly valuable nutritionally and tasteful meat, and wool is used for the manufacture of consumer textiles, skins - for the manufacture of sheepskins and sewing high-quality sheepskin. Some assortments of elastic semi-thin wool - an indispensable raw material for the manufacture of fire-resistant seats of motor vehicles, are in great demand among well-known automakers (*Islamov, 2011a,b*). In modern market conditions, it is necessary to pay due attention to the breed resources of sheep of the intensive type, which are characterized, first of all, by high genetically determined fecundity in combination with diversified productivity. With increased fecundity of the queen and the growing of a large number of young stock, the necessary prerequisites are created for carrying out more rigorous selection and accelerating the change of generations, which undoubtedly is reflected in the increased effect of selection. The high fecundity of females and the preservation of young stockings create the necessary prerequisites for increasing the production of mutton and other sheep products. An important reserve in the production of lamb in fine-wool sheep breeding is industrial crossing with sheep of domestic half-fine breeds: Kazakh meat and wool half-breeding, with sheep of three intrabreed types: Axenger, Chuysky and Kalchengilsky types, with sheep of three breed types: Axenger, Chuysky and Chelighilsky types, with the Demersky type of sheep and other breeds of the breeding type: Axeringa, Chu and Kalchengil; meat and meat-wool breeds

(Islamov *et al.*, 2015). The use in industrial crossing is of greater importance in increasing the meat productivity of fine-fleece and fine-wool-coarse-wool sheep wool meat and sebaceous breeds and, above all, rams of the Edilbayev breed. The Research Institute of Sheep Breeding is a branch of the Kazakh Research Institute of Livestock and Feed Production in this direction conducts promising research work on the creation of herds of fine-fleeced sheep with three zonal types based on domestic genotypes (SKM, NKM, KT) and foreign breeds (AVM), with fineness of wool within 19-21 microns and productivity corresponding to world analogues - trimmed wool of females in breeding herds 3.0-4.0 kg, live weight 55-60kg. The productivity of sheep of the desirable types exceeds the standards of analogues in live weight by 15–22.0% and the trimmed wool washed by 20.0–25.0%.

Semi-fine sheep breeding

In Kazakhstan, new breeds of meat-wool semi-fine-wool breeds have been created, giving semi-thin wool, including a crossbred and crossbred type, and a large amount of mutton. These are Kazakh meat and wool with three intrabreed types (Achenger, Chui, Kalchengil), Akzhaik meat and wool, and Kazakh half-fleece, both with crossbred wool, Degerssian meat-wool, Kazakh half-fine meat sheep of the type Hampshire, Kazakh pattern, such as Hampshire, Kazakh pattern. Wool of domestic breeds should be ensured by improving its quality in purebred breeding and use on sheep and meat wines, Kazakh semi-fine and Akzhaik breeds as far as possible. Imported sheep of English long-haired breeds, in particular, Lincoln sheep, Romney-march and some other breeds. In Kazakhstan, scientists and practitioners-sheep breeders by long selection in 2011 tested a multi-breed intra-breed type of Kazakh meat-wool breed. The type was created on the basis of the crossing of Kazakh fine-woolited queens with sheep Finnish landrace and using on the queens of the desired type of Kalchengil sheep in the southeast zone and crossing the queens of the Chuysky type MSK with the sheep Finnish landras in the southern zone. The number of multiple types is more than 3 thousand heads. Fecundity of females - 140-160%. 3 factory highly productive lines (combined, thick-haired and multi-fruited) of Kazakh meat and wool breed with a live weight of 55.0-60 kg and a cut of 2.0-2.3 kg of wool were created, as well as a line of Kazakh semi-fine wool with crossbred wool (live weight 55-60 and cut wool (2.3-2.4 kg) (Islamov *et al.*, 2016; Islamov *et al.*, 2018). The prospect of increasing the production of meat from semi-fine sheep along with the increase in livestock should be ensured by improving their productive qualities, with purebred breeding and based on interbreeding with using the following early imported breeds: Finnish landrace, polypos - to increase fertility in Kazakh multiple babies up to 180-200% and to create highly productive herds of sheep with fertility 170-175% on the basis

of Kazakh fine-fleeced queens, meat and slaughter qualities of the Kazakh sheep of the Hampshire type and the creation on this basis of new herds of early-ripening meat sheep. Of the total number of sheep (18.5 million goals), the proportion of breeding animals is 5.3%. For the successful development of sheep breeding and ensuring its competitiveness in a market economy, it is necessary to bring the proportion of breeding sheep to 15-20% of the total number of sheep. Thus, the main areas of breeding work in sheep breeding in Kazakhstan are aimed at improving the existing fine-wool, semi-fine-brown and meat-tallow breeds sheep and the creation of new highly productive breeds, types, lines in order to obtain competitive products (*Alzhaxina et al., 2014*). Analysis of the state of breeding and breeding work the past few years shows that tangible progress in increasing the proportion of breeding sheep is also not observed. One can only note an increase in the number and stabilization of the livestock of sheep (perhaps optimal for the republic) at the level of 18-20 million, since the proportion of breeding animals is only 5.3%. And what is the way, how and when it is possible to achieve the proportion of breeding animals at the level of 15-20% from the analysis of scientific publications remains unclear. It should be noted that until 1990 the specific weight of breeding sheep was 50%. If we refer to the Statistics Agency of the Republic of Kazakhstan, then, as in Soviet times, only the number of sheep is known, sheathed wool on average throughout the population, not even areas of sheep, not to mention cutting in the context of individual breeds. The reason is in the absence of a proven methodology for analyzing qualitative indicators of productivity growth, and the available data on scientific publications are reduced, as a rule, to the characteristics of individual herds, lines, and breeding groups and cannot characterize comprehensive assessments for the whole breed. As a result, it is difficult to determine at what price and how much fine quality wool, semi-thin, including crossbred, was sold. Therefore, the hard, painstaking work of breeders who have achieved high levels, reflecting this analytical review, is “sinking” and “bleaching” in imperfect socio-economic and regulatory conditions. Indeed, there are questions about who and how should regulate the prices of products, who and how should change the method of compiling statistics, taking into account primarily the identification of quality indicators of productivity, not limited to only quantitative indicators. Sheep breeding at the present stage of development, says the “Concept for the development of livestock industries in Kazakhstan”, is characterized by small marketability, which, combined with a significant decrease in its highly productive breeding part, during periods of privatization of livestock of public farms led to a deterioration in the quality of products, mainly sheep. Meanwhile, the prospect of further development of sheep breeding predetermines the need to ensure competitive products (*Islamov et al., 2015*). The state policy in this aspect should be aimed at: bringing the livestock of sheep to 22-23 million by

2025. and the proportion of breeding sheep - up to 15-20% of their total number; production of wool in physical mass - up to 50- 46 thousand tons, including scoured - up to 20-22 thousand tons; ensuring the breed zoning of sheep on the basis of the State Register of Breeding Achievements in Livestock; implementation of the harvesting, processing and marketing of products based on multifunctional clusters, bringing the sale of wool on the foreign market to 10-13 thousand tons; the use of highly productive breeding animals by leasing; harmonization of national standards (CIS) with international standards (ISO); a gradual transition from small-scale to large commodity agricultural formations in order to improve quality and reduce production costs, restore mobile sheep breeding and efficient seasonal use of distant and mountain pastures; - bringing the production of sheep milk from 24 up to 50 thousand tons. Considering the problems and prospects for the development of Kazakhstan's sheep farming, it should be noted, the authors argue that in conditions of small their peasant (farmer) farms, it is impossible to conduct purposeful breeding work, since sheep are usually kept together with their queens all year round. Moreover, such farms are not able to acquire high-quality producers to improve their herds. World experience shows that sheep breeding is beneficial if at least 3,000 sheep are raised in one farm. Therefore, small peasant (farm) farms should be merged into larger sheep-breeding formations, as it was before in the republic, and as is the case in all sheep-breeding countries of the world. In many regions of Kazakhstan, 4-5 sheep breeds of different directions of productivity are bred simultaneously. Thus, improving the breeding zoning of sheep in the republic and ensuring its legislative compliance will allow breeding the most productive and localized sheep, which will contribute to increasing the efficiency of the industry. Improving the breeding work and preserving the gene pool of animals will further continue on a large scale breeding of breeds and types of animals with higher productivity, better quality products that meet market needs, a high level of transformation of plant products into animal products. Increasing the export potential of livestock in the country is one of the key priorities of the state policy of the Republic of Kazakhstan. The creation of a factory type of meat early ripened semi-coarse sheep is a priority and timely direction in sheep breeding. Important reserve in manufacturing competitive lamb in sheep fine industrial crossing is based on the use of domestic sheep half-fine in type Hampshire, MSHK, degeresskaya and import meat and meat-wool breeds - doychemerinofleyshaff, Rambouillet, Australian Merino meat and Dona, Finnish Landrace half-fine - suffolk, polypus, Hampshire, Oxforddown, Southdown and others. In connection with the entry of Kazakhstan into the customs union with Russia and Belarus, as well as with entry into the WTO, it is necessary to reorient some types of products, taking into account its relevance in the countries of near and far abroad, first of all, of the European continent. Taking into account that

sheep of meat and greasy areas take the first place (over 70% of the total livestock) in the country, it is necessary to create types and lines of these sheep differing in a lower proportion of fat in meat-pulp and marbling of meat.

As a result of purposeful, scientifically based breeding, and jointly with specialists from farms in southern Kazakhstan, research has been conducted on the creation of the Ordabasy fat-tailed breed of sheep of meat and fat productivity. It was created by complex reproductive cross-breeding of ewes of the local Kazakh fat-tailed coarse-haired breed with sheep edibayev (domestic) and gissar (foreign) sheep breeds, with subsequent breeding of desirable types of "in itself" with a live weight of producing sheep - 110-123 kg, queens - 68 -80 kg, baranchik to beating off - 33-43 kg. The breed has 6 lines. In the Eastern, South-Eastern and Western regions of the country - in the herds of the breeding plant them. The Kurmangazy Atyrau, farm "Mashan" of Almaty and the Kokzhyra of the East Kazakhstan regions created a rather large array of fat-tailed sheep of a new type - with white and light gray wool, combined with high meat-fat qualities and distinguished by their exceptional adaptability to the conditions of breeding in the desert, semi-desert and foothill-steppe zones. The total number of sheep of a new type is 10.2 thousand heads, including queens 6.2 thousand heads. The average live weight of sheep of new-type producers, depending on the breeding zone, varies within 93.6-103.4 kg, or higher than the requirements of the standard of Kazakh sheep coarse-haired breed for the corresponding group of animals by 17.0-29.2% , queens - 66.7-70.6 kg and 11.2-17.6%, respectively; 1.5-year-old rams - 73.5-78.3 kg and 13.1-20.4% and 1.5 -year bright - 55.8-58.2 kg and 11.6-16.4%. The new intrabreed type of sheep of the Kazakh fat-tailed coarse-haired breed was successfully tested and a patent was obtained for the selection achievement of the Republic of Kazakhstan. The main difference between sheep of the new type is their coarse wool of white and light gray color, which is in high demand among the population and enterprises of the country's light industry. She goes for making felt mats, carpets, home decoration, knitted coarse-woven products, and the sheepskin of these sheep for making elegant and light sheepskin coats. The lightened wool of new type of coarse-haired coarse sheep differs in physical and mechanical properties in comparison with the Kazakh fat tail coarse-haired sheep. Their wool can be attributed to a new variety of coarse wool of sheep bred in Kazakhstan, it has better technological properties compared to the wool of fat coarse-haired sheep. In the wool of fat-tail coarse-haired sheep of a new type compared to the standard, there are much less downy (depending on the age and age groups 6.1-12.2%), more - fine (0.8-5.8%) and medium (0.9-2.8%) core fibers, which indicates a change in the composition of the forming fibers in the desired direction . Such wool is closer in quality to carpet wool. The content of transitional fibers in the wool of the

studied groups of sheep is about normal (rams are 1.8% higher), coarse guard lines - 0.6-3.6% higher.

Conclusion

ROC breeding should take its rightful place in the agro-industrial complex of the country, become one from promising sectors of domestic livestock farming, providing the population of the country with essential foodstuffs, and light industry with raw materials, based on the rational use of the genetic potential of existing and newly created breeds, types and lines of sheep in various directions productivity. The role of sheep farming in raising welfare, especially of the rural population and, in general, the overall socio-economic situation of the republic is unquestionably large and has all the prerequisites for successful development.

Stanje i perspektive razvoja ovčarstva u Kazahstanu

Yessenbay I. Islamov, Gulzhan A. Kulmanova

Rezime

Rad prezentuje stanje i perspektive razvoja ovčarstva u Kazahstanu. U radu su razmatrana pitanja sadašnjeg stanja i daljeg razvoja odgoja ovaca u Kazahstanu. U konceptu svrstavanja Republike Kazahstan među 30 najrazvijenijih država sveta, potrebno je rešiti niz problema u najperspektivnijim oblastima, od kojih je jedan prelazak na inovativne pravce poljoprivrednog sektora zemlje, uvođenje visoko efikasnih tehnologija i rad na osnovu najboljih međunarodnih standarda. Pod tim uslovima, vodeću ulogu u rešavanju problema igra intenzivni razvoj ovčarstva, koji populaciji pruža potrebe za specifičnim vrstama sirovina i hrane. Ovčarstvo je važna grana stočarstva. Za razliku od mnogih drugih domaćih životinja, ovce daju najveću količinu raznih proizvoda: vune za razne svrhe, mleko, ovčje krzno i kožu. Lista proizvoda proizvedenih od proizvoda dobijenih od ovce još je obimnija: tkanine i pletiva, krzno i kožni proizvodi, brojni prehrambeni proizvodi. Takvu raznolikost proizvoda i sekundarnih proizvoda pruža veliki broj rasa ovaca.

Ključne reči: rasa, genski fond stada, linija, selekcija i odgajivanje

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SOME NON-GENETIC FACTORS AFFECTING LAMBS BIRTH WEIGHT IN F1 GENERATION OF MIS X ILE DE FRANCE

Violeta Caro Petrovic¹, Milan P. Petrovic¹, Marina I. Selionova², Dragana Ružić-Muslic¹, Nevena Maksimovic¹, Bogdan Cekic¹, Ivan Pavlović³

¹Institute for Animal Husbandry, Belgrade-Zemun, Serbia

²Institute for Sheep and Goat Breeding, Stavropol, Russia

³Scientific Veterinary Institute of Serbia, Belgrade, Serbia

Corresponding author: Violeta Caro Petrovic, violycaro@yahoo.com

Invited paper

Abstract: The study aim was to know the non-genetic factors and the interaction effect on birth weight of lambs F1 generation crossed Mis x Ile de France. The data of lambs birth weight (in kg), month of birth, year of birth, season of birth, sex, birth type from progeny (F1 generation) of crossing Mis x Ile de France with a total of 388 lambs born during 2018 to 2019 recorded. The General Linear Model (GLM) procedure was used to analyze the data. The analysis of variance has shown that month of birth significantly affects lambs birth weight ($P < .005$). Results shows a highly significant effect of year on lambs birth weight ($P < .001$). The highest mean average birth weight of lambs showed on the second year. Regarding of season, lambs had the highest body weight in spring and winter, and the smallest in autumn but despite the differences in weight, season did not show any significant effect on lambs birth weight ($P > .005$). Sex of lambs showed no significant effect on lambs birth weight ($P > .005$). The birth type significantly affects birth weight of lambs ($P < .001$). The highest body weight are have single, then twins, triplets and quadruplets.

Key words: crossbreeding, non-genetic factors, lambs, birth weight

Introduction

The birth weight is the first observed trait in the life of an animal of which growth, production, and reproduction, are dependent (*Thiruvankadan et al., 2008*). Likewise, the birth weight of lambs has an essential role in satisfying sheep production (*Caro Petrovic et al., 2013*). The birth weight of lamb has highly

influenced by both genetic and non-genetic factors. The understanding of the non-genetic factors which influence the development and growth of lambs may help for changes in the breeding plans and management practices to minimize the influence of factors which reduce production performance (*Petrovic, 2000; Siddalingamurthy et al., 2017*). Birth weight as an early measurable trait is of great interest because of its positive genetic correlation with further live weights (*Mellado et al., 2016*). The non-genetic factors must be corrected before starting a genetic analysis. In several studies, the effect of non-genetic factors on growth performance in sheep has been investigated (*Tohidi et al., 2016*). Environmental factors such as age, sex, type of birth, and year of birth influenced the estimation of breeding value (*Momoh et al., 2013*). Body weight of the lambs at birth has an important role in achieving a better sheep production (*Petrović et al., 2011*). By knowing the factors affecting the birth weight, it will lead changes in breeding and management schemes (*Caro Petrovic et al., 2013*). For several years, it has identified that in lamb production programs, body weight at birth of lambs depends on the genetic type, sheep age, sex, type of birth (single vs. multiple), season and year of birth are factors exert influence on sheep growth (*Catalan, 2018*)

The study aim was to know the non-genetic factors and the interaction effect on birth weight of lambs F1 generation crossed Mis x Ile de France.

Materials and Methods

The data of lambs birth weight (in kg), month of birth, year of birth, season of birth, sex, birth type from progeny (F1 generation) of crossing Mis x Ile de France with a total of 388 lambs born during 2018 to 2019 recorded at the experimental farm of the Institute of Animal Husbandry were utilized in this study. All animals had the same diet and care conditions. The General Linear Model (GLM) procedure of the SPSS version 20 was used to analyze the data. The following mathematical procedure was applied.

$$Y_{ijklmn} = \mu + M_i + Y_j + S_k + G_l + T_m + e_{ijklmn}, \text{ where:}$$

Y_{ijklm} - birth weight of lambs of m birth type, l sex, k season, j year, i month

μ - Overall population mean

M_i - effect of month

Y_j - effect of year

S_k - effect of season

G_l - effect of sex

T_m - effect of birth type

e_{ijklmn} - other undetermined impacts

Based on the results obtained, certain conclusions were drawn and a discussion was conducted.

Results and Discussion

The birth weight of lambs on the month of birth showed that most lambs were born on March, having the lowest and the highest birth weight while the least born lambs were on April born (Table 1).

Table 1. The average birth weight of lambs, standard deviation, standard error of mean and variances in four different month

Month of birth	Mean	N	Std. Deviation	Std. Error of Mean	Minimum	Maximum	Variance
January	4.86	59	.891	.116	3.10	6.50	.794
March	4.44	237	.976	.063	2.10	6.80	.953
April	4.67	23	.784	.163	3.60	6.10	.614
October	4.30	69	.940	.113	2.50	6.70	.883
Total	4.48	388	.962	.048	2.10	6.80	.925

The highest mean average birth weight of lambs showed on the month of January, and the lowest average birth weight was on the month of October. The differences for the average of birth weight were 0.42kg, 0.19kg, and 0.56kg between January to March, January to April and January to October month of lambs' birth.

The analysis of variance has shown that month of birth significantly affects lambs birth weight ($P < .005$).

The effect of year on lambs birth weight performances have shown in table 2.

Table 2. Average birth weight, standard deviation, standard error, variance of weight of lambs F1 generation of Mis x Ile de France per year

year	Mean	N	Std. Deviation	Std. Error of Mean	Minimum	Maximum	Variance
1.00	4.3180	228	.97767	.06475	2.10	6.70	.956
2.00	4.7206	160	.88929	.07030	2.50	6.80	.791
Total	4.4840	388	.96175	.04883	2.10	6.80	.925

In table 2 results show a highly significant effect of year on lambs birth weight ($P < .001$). On the second year have a higher mean average birth weight of lambs in comparison on the first year. The difference in average birth weight was 0.40 kg between year 2 and year 1.

The season is one of the important factors affecting the body of animals and therefore the body weight of lambs at birth. Results of Average birth weight of lambs, standard deviation, standard error of mean during winter, spring and autumn are presented in the next table.

Table 3. Average birth weight of lambs, standard deviation, standard error of mean during winter, spring and autumn

season	BWB Mean	N	Std. Deviation	Std. Error of Mean	Minimum	Maximum	Variance
Winter	4.5271	166	.98832	.07671	2.10	6.70	.977
Spring	4.5392	153	.93339	.07546	3.00	6.80	.871
Autumn	4.2580	69	.93987	.11315	2.50	6.70	.883
Total	4.4840	388	.96175	.04883	2.10	6.80	.925

The lambs had the highest body weight in spring 4.53 kg and winter 4.52 kg, and the smallest in autumn 4.25kg. However, despite the differences in weight, season did not show any significant effect on lambs birth weight ($P > .005$).

Table 4. Effect of sex on average birth weight of lambs

sex	BWB Mean	N	Std. Deviation	Std. Error of Mean	Minimum	Maximum	Variance
Male	4.50	189	.948	.069	2.50	6.80	.900
Female	4.46	199	.976	.069	2.10	6.70	.953
Total	4.48	388	.962	.048	2.10	6.80	.925

In the table above clearly seen that there is a mild difference of 0.04kg in body weight at birth between the male and female lambs F1 generation of Mis x Ile France. Result in this study showed that sex of lambs no significant effect on lambs birth weight ($P > .005$). Unlike with ours, the research study by *Rahimi et al. (2014)* their results showed that non- genetic factors have an important role in expressing of genetic potential in the lambs

that male lambs were heavier than females and their difference was significant ($P < 0.01$).

Table 5. Effect of birth type on average birth weight of lambs

Birth type	Mean	N	Std. Deviation	Std. Error of Mean	Minimum	Maximum	Variance
Single	5.39	117	.877	.081	2.60	6.80	.770
Twin	4.25	215	.624	.043	2.50	6.10	.390
Triplet	3.52	52	.564	.078	2.50	5.10	.318
Quadruplet	2.75	4	.519	.259	2.10	3.30	.270
Total	4.48	388	.962	.048	2.10	6.80	.925

The birth type significantly affects the birth weight of lambs F1 generation of Mis x Ile de France ($P < .001$). One can see the biologically expected difference in lamb weight depending on the type of birth. The highest body weight has seen on single, then twins, triplets and quadruplets. Similar results have presented, by *Gbangboche et al.*, (2006). By them, single lambs and male lambs were heavier than those twins and females. Lambs that are born singles have 9.6% higher birth weight compared with twins (*Simeonov et al.*, 2015).

Our results of birth weight have confirmed by some authors of such *Ebangi et al.* (1996) stated that their results indicate that breed and non-genetic factors significantly affect birth weight and by *Mellado et al.* (2016) litter size was the most important factor affecting birth weight of lambs. *de Combellas et al.* (1980) found a significant effect of year on birth weight.

Other scholars mildly agree and disagree with the result we have obtained. *Siddalingamurthy et al.* (2017) the year of birth of lamb had a non-significant effect on the birth weight. The season of birth of the lamb, sex of the lamb significantly affects the birth weight. The mean birth weight of different sexes revealed that the male lambs had significantly ($P \leq 0.01$) higher birth weight. In the study of *Thiruvengadan et al.* (2008) the birth weight was highly significantly ($P < 0.01$) affected by the period of birth, the season of birth, sex of the lamb (*Fasae et al.*, 2012). *Marufa et al.* (2017) the birth type, sex and year had significant ($p < 0.05$) effect on birth weight. *Catalán et al.* (2018) concluded that the effects of year and season of birth, parturition number, type of parturition and sex, had a significant effect on lamb birth weight. Many other authors attach great importance to the influence of paragenetic factors on the weight of lambs (*Fasae et al.*, (2012); *Momoh et al.*, 2013; *Caro Petrović et al.*, 2013; *Mellado et al.*, 2016).

Conclusion

The month of birth, year of birth, the type of birth significantly affected the birth weight of the F1 generation lambs of Mis x Ile France. However, the season of birth and sex of lambs have no effect on the birth weight of lambs F1 generation of Mis x Ile de France.

Neki negenetski faktori koji utiču na porođajnu masu jagnjadi u F1 generaciji Mis x Ile de France

Violeta Caro Petrovic, Milan P. Petrovic, Marina I. Selionova, Dragana Ružić-Muslic, Nevena Maksimovic, Bogdan Cekic, Ivan Pavlović

Rezime

Cilj istraživanja bio je spoznati uticaj interakcije negenetske faktore na masu jagnjadi pri rođenju. Analizirani su podaci o masama janjadi (u kg), F1 generacije Mis x Ile de France. Observirani su sledeći negenetski faktori: mesec rođenja, godina rođenja, sezona rođenja, pol, tip rođenja kod ukupno 388 janjaca rođenih tokom 2018. i 2019 godine. Za analizu podataka korišćen je postupak Generalnog Linearnog Modela (GLM). Analiza varijanse pokazala je da mesec rođenja značajno utiče na masu kod rođenja janjadi ($P < .005$). Rezultati pokazuju veoma značajan uticaj godine na masu rođenja janjadi ($P < .001$). Najveću srednju prosečnu masu imala su jagnjad u drugoj godini. Sa aspekta uticaja godišnjeg doba, jagnjad su imale najveću telesnu masu u proleće i zimi, a najmanju u jesen, ali uprkos razlikama u masi, sezona nije pokazala značajan uticaj na masu janjadi ($P > .005$). Pol jagnjadi nije pokazao značajan uticaj na masu janjadi ($P > .005$). Tip rođenja značajno utiče na masu jagnjadi kod rođenja ($P < .001$). Najveću telesnu masu imaju jedinci, zatim blizanci, trojke i četvorke.

Ključne reči: ukrštanje, negenetski faktori, jagnjad, masa rođenja

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GROWTH RATE OF LOCAL PIG BREEDS: STUDY OF PROJECT TREASURE

Marjeta Čandek-Potokar^{1,2}, Nina Batorek Lukač¹, Urška Tomažin¹, Rosa Nieto³

¹ KIS (Agricultural Institute of Slovenia), Hacquetova ul. 17, 1000 Ljubljana, Slovenia

² University of Maribor, Faculty of Agriculture and Life Sciences, Pivola 10, SI-2311 Hoče, Slovenia

³ CSIC, EEZ, Profesor Albareda s/n, 18008, Granada, Spain

Corresponding author: Marjeta Čandek-Potokar, meta.candek-potokar@kis.si

Invited paper

Abstract: The information on average daily gain (ADG) according to production stage or growing phase was collected for 20 local European pig breeds. At the end of lactation, the ADG of local pig breeds is similar as in modern genotypes, but the lactation period is longer and the weight of piglets at birth is lower. At the end of the early growth (post-weaning) period, local pig breeds demonstrate a bit lower ADG than modern genotypes, whereas their growth rate in the fattening phase is considerably lower than in modern breeds, in particular in late fattening phases. The study demonstrated a great variability in data, in terms of availability and quality of information, and the reported growth rates reflect the heterogeneity of different production systems and feeding regimes used. Majority of studies simulated practical conditions of production, and only a few allowed a rough estimation of the growth potential of local pig breeds, an important information to estimate the nutritional needs of the animals and to allow an optimal utilisation of local breeds of pigs.

Key words: pig, performance; growth rate; local breed

Introduction

Knowledge about growth rate of local pig breeds is very limited. In particular, there is a lack of information about their growth potential at different stages of development, which would allow to assess their nutritional needs and to optimize the production. Most of the information available concerns Iberian pigs, for which the optimal ratios between protein and dietary energy have been studied (*Nieto et al., 2012*) and their specific metabolic characteristics compared to modern conventional pig breeds (*Fernández-Fígares et al., 2007*). Although knowledge about nutritional requirements of Iberian pigs, as representative of breeds not

submitted to selective breeding for lean growth, may be indicative for other local pig breeds, it does not allow direct extrapolation. This study consists of a review of available information on growth performance of local pig breeds considered in the project TREASURE and a comparative analysis of the collected information as a first step in assessing the growth potential and nutritional requirements of these animals.

Material and Methods

The objective of this analysis was to assemble the growth data for all the breeds studied in the project, at different stages of fattening. Data on average daily gain (ADG) were collected from the available literature (articles, theses, congress proceedings) or recorded in the experiments conducted as part of the project. The objective was to evaluate breeds in their current phenotype and not based on historical data. Thus, only recent studies (up to 20 years) have been taken into account (Table 1).

Table 1. Number of recorded data according to breed and growth phase

Local pig breed	Country	Lactation	Post-weaning	Fattening			
				I	II	I+II	III
Apulo Calabrese	Italy	3	2	3	3	4	3
Alentejana	Portugal	11	1	8	17	28	4
Basque	France		3	4	4	7	4
Bísara	Portugal	3	2	3	5	13	1
Casertana	Italy			2	2	5	2
Cinta Senese	Italy	12	4	3	1	15	5
Black Slavonian pig	Croatia	3	1			19	1
Gascon	France			5	3	14	4
Iberian pig	Spain	14	13	8	9	27	34
Krškopolje pig	Slovenia	3	4	6	6	9	5
Old type Lithuanian white	Lithuania	3		2	2	3	
Lithuanian indigenous wattle	Lithuania	5	2	2	2	3	
Mangalitsa (Swallow bellied)	Serbia	9	2	1	1	9	1
Moravka	Serbia	2	1	1	2	8	1
Mora Romagnola	Italy			1	1	2	1
Black Majorcan pig	Spain	1	1	1		2	2
Nero Siciliano	Italy			3	5	15	
Sarda	Italy		5	3	3	11	6
Schwäbisch Hällisches	Germany	7	7	1		10	1
Turopolje pig	Croatia	1				3	

We defined the phases very roughly because of the large differences in weight range between studies. The ADG of the piglets during lactation was taken as reported and regardless of the duration of lactation (information on the duration of lactation was not always provided). The studies considered were those that reported live body weight (BW) or ADG for

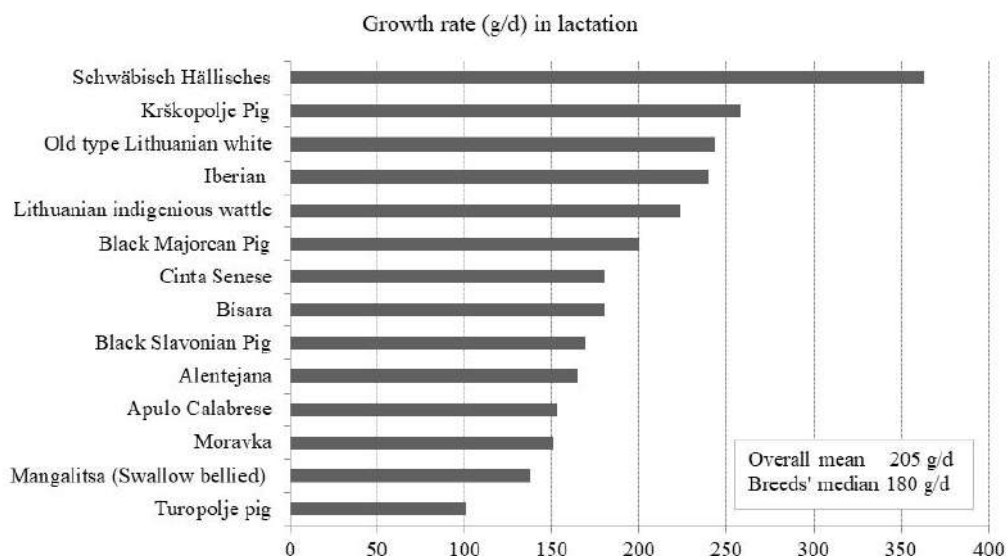
- a) post-weaning phase between weaning and approximately 30 kg of BW,
- b) fattening phase I; approximately from 30 to 60 kg BW,
- c) fattening phase II; approximately from 60 to 100 kg of BW,
- d) fattening phase III; above 100 kg of BW.

Sometimes the data source provided only the overall ADG for the entire studied period, or the ADG could be calculated from the BW data. The unit of observation was either a study or an experiment or a part of the experiment (e.g. growth stage, diet), depending on the experimental design of the study. When only one data source was available, it was considered representative of the breed. For data analysis, the UNIVARIATE procedure of the SAS® software (v9.4, SAS Inst Inc., Cary, NC) was used to compute basic statistical parameters.

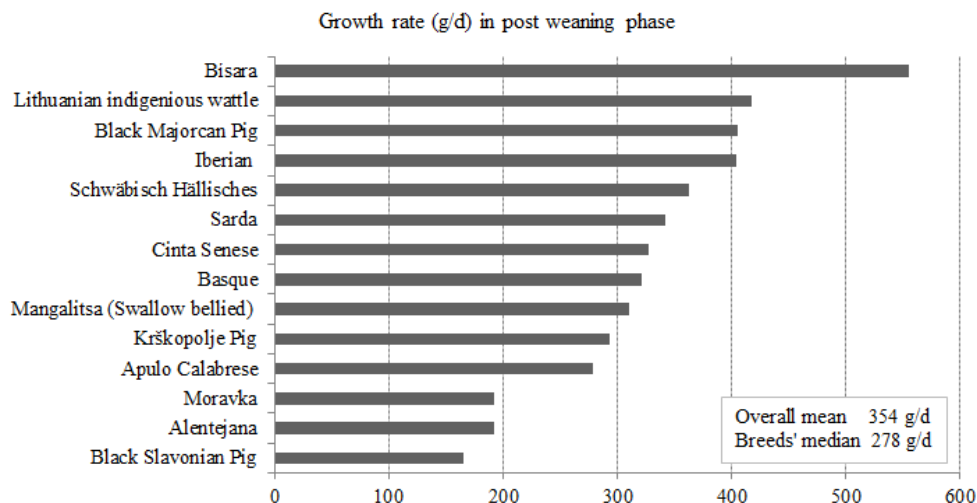
With regard to the level of feed associated with the growth data, the information on the correspondence between the reported and expected feed intake (see Čandek-Potokar *et al.*, 2019) allows to assume that pigs in the surveyed studies were mainly fed ad libitum in the post-weaning and early fattening phase, whereas in the late fattening phases their feeding was mostly restricted. There was a great variability among breeds regarding data availability as well as reported results. Moreover, the results were obtained under very different feeding and management conditions. For these reasons, it was very difficult to establish a harmonized approach to data analysis and comparison. As a result, the analysis is mainly descriptive and based on basic statistical parameters, however allowing to draw some interesting observations and conclusions.

Results

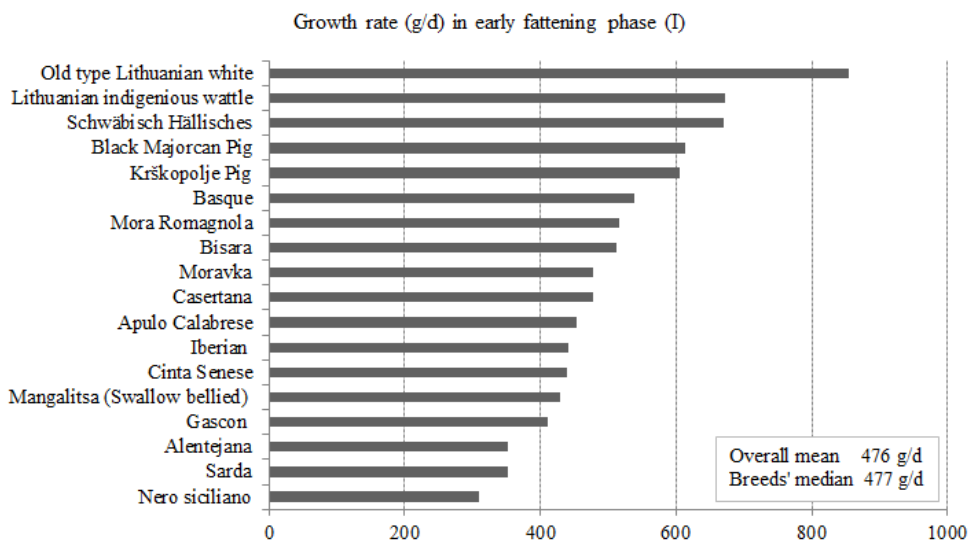
The average growth rates in different production phases calculated for breeds are presented in Figures 1-5. The ADG during lactation could be recorded for 14 out of 20 breeds. The overall average was 205 g per day (g/d) and ranged from 101 g/d (Turopolje pig) to 363 g/d (Schwäbisch Hällisches pig). The median value for breed averages was 180 g/d.



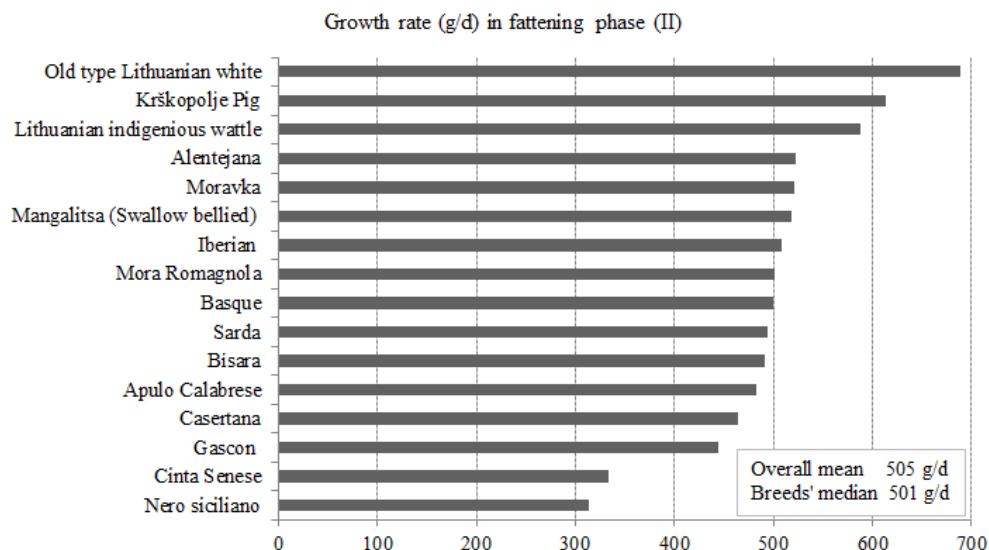
During the post-weaning phase (information available for 14 out of 20 breeds), the overall ADG was 354 g/d and ranged from 165 g/d (Black Slavonian Pig) to 555 g/day (Bísaro pig). The median value for breed averages was 278 g/d.



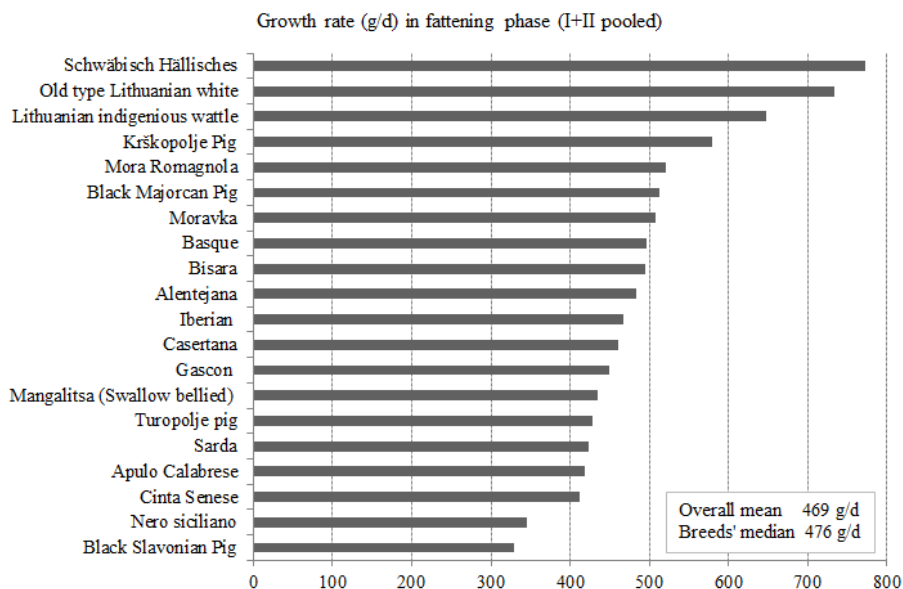
For the early fattening phase (I; approximately 30-60 kg of BW), information was available for 18 breeds and the overall average ADG was 476 g/d, ranging from 310 g/d (Nero Siciliano pig) to 855 g/d (Old type Lithuanian white). The median value for breed averages was 477 g/d.



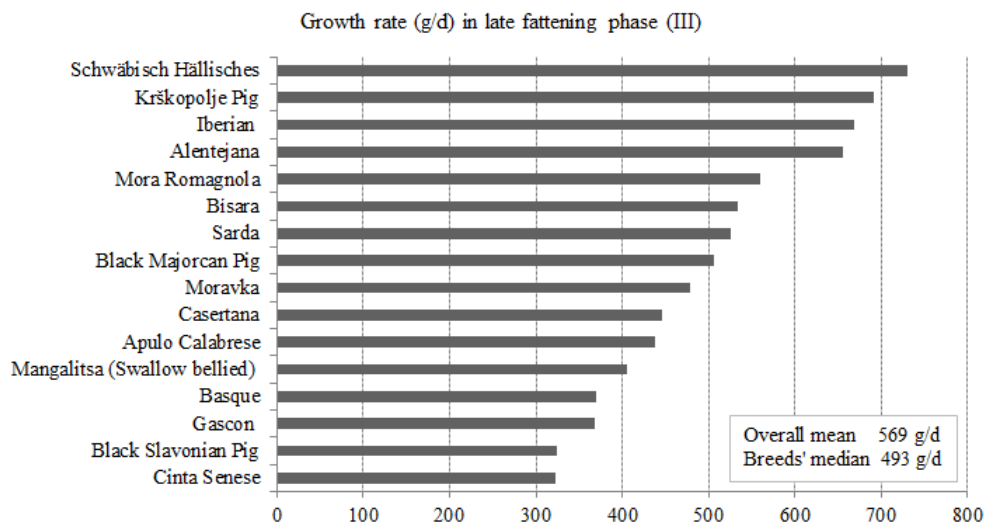
For the fattening phase II (about 60-100 kg of BW), data were found for 16 breeds; the overall ADG was 505 g/d and ranged from 314 g/d (Nero Siciliano pig) to 689 g/d (Old type Lithuanian white). The median value for breed averages was 501 g/d.



The overall ADG for pooled fattening phases I and II (30-100 kg of BW) was 469 g/d and ranged from 329 g/d (Black Slavonian pig) to 773 g/d (Schwäbisch Hällisches pig). The median value for breed averages was 476 g/d.



Data for the late fattening phase (III; above 100 kg of BW) were available for 16 breeds; the overall average was 569 g/d and the ADG ranged from 322 g/d (Cinta Senese pig) to 730 g/d (Schwäbisch Hällisches pig). The median value for breed averages was 493 g/d.



Discussion

Despite a longer lactation period practiced in local pig breeds (32 to 64 days; in average 47.2 ± 9.0 days; see Čandek-Potokar *et al.*, 2019), reported growth rates of piglets in lactation are generally slightly lower compared to modern breeds reared in intensive systems (Quiniou *et al.*, 2002). Pre-weaning growth is dependent on sow milk production and sow management during lactation (Koketsu *et al.*, 2017). It can therefore be assumed that the observed results reflect the management of the sows as milk production is positively and significantly related to piglet's weight (Etienne *et al.*, 2000). However, studies conducted on the Iberian breed suggest that weaker ADG compared to modern breeds could also be related to a lower efficiency in the use of milk nutrients (Aguinaga *et al.*, 2011).

Based on the correspondence between the reported and expected feed intake in the post-weaning phase (see Čandek-Potokar *et al.*, 2019), it appears that during this phase the pigs were mainly fed ad libitum; the reported ADG would therefore indicate growth potential. Nevertheless, the overall ADG (354 g/d)

appears slightly lower than what is reported for modern breeds in intensive systems (Magowan *et al.*, 2007; Collins *et al.*, 2017). The maximum values observed among the collected data would indicate that the growth potential of local pig breeds at this stage might be higher. In this context, the comparison with results obtained in Iberian pig under controlled experimental conditions is interesting. For Iberian pig, the average calculated from the literature was 404 g/d, which is similar to the ADG achieved under controlled conditions (416 g/d, Conde-Aguilera *et al.*, 2011), and slightly lower than in modern breeds at comparable BW. Even at this stage, an increased predisposition for fat tissue gain has been demonstrated in the Iberian pig (Conde-Aguilera *et al.*, 2011).

The fattening phase is characterized by a much lower ADG in local breeds than in genetically improved breeds, for which it is well known that they can reach ADG above 1000 g/d in intensive systems. Moreover, the data show high heterogeneity, not only between breeds, but also within data of the same breed, which can be explained by differences between studies in dietary intake levels and/or conditions. It is interesting to look at the extreme values as they could indicate the growth potential of the breed. In the case of Iberian pigs, for which there are studies evaluating growth potential in almost *ad libitum* conditions (Nieto *et al.*, 2002; Barea *et al.*, 2007; García-Valverde *et al.*, 2008) the reported ADG values are higher than those reported in this study (i.e. 559 g/d, 854 g/d and 918 g/d for the 25-50 kg, 50-100 kg and 100-150 kg periods, respectively). Another observation concerns the particular case of Iberian and Alentejano pigs, which demonstrate high ADG in the late fattening phase. This results from their specific production system, namely a restricted feeding up to 80-100 kg BW, followed by *ad libitum* feeding in the montanera system (compensatory growth phase, García-Casco *et al.*, 2015; Freitas, 1998) when pigs are outdoors and feed exclusively on acorns and pasture. The opposite is the case in other breeds, where it is usual to observe a similar ADG in the early and late stages or a lower ADG in the late stage of fattening (Figure 2), consistent with feed intake data indicative of restricted feeding in this phase (Čandek-Potokar *et al.*, 2019).

Conclusions

For many local pig breeds the information collected provides a preliminary estimation of their growth potential. Despite the limitations of this study, some interesting observations could be drawn, showing knowledge gaps as well as a need for more advanced research to better characterize the nutritional needs and growth potential of local pig breeds in order to optimise their use and contribute to their future development and preservation.

Stopa rasta lokalnih rasa svinja: studija projekta TREASURE

Marjeta Čandek-Potokar, Nina Batorek Lukač, Urška Tomažin, Rosa Nieto

Rezime

Informacije o prosečnom dnevnem prirastu (DP) prema fazi proizvodnje ili tova prikupljene su za 20 lokalnih evropskih rasa svinja. Na kraju laktacije, DP lokalnih rasa svinja je sličan kao kod modernih rasa, ali je period laktacije duži, a težina prasadi pri rođenju niža. Nakon odbijanja, lokalne rase svinja pokazuju nešto niži DP od modernih rasa, dok je njihova stopa rasta u fazi tova znatno niža nego kod savremenih rasa, posebno u kasnim fazama tova. Studija je pokazala veliku varijabilnost podataka, u smislu dostupnosti i kvaliteta informacija a isto tako i samih rezultata prirasta, koji odražavaju heterogenost različitih proizvodnih sistema i režima ishrane. Većina studija simulirala je praktične uslove proizvodnje, a samo nekoliko njih je omogućilo grubu procenu potencijala rasta lokalnih rasa svinja, što je važna informacija za procenu prehrambenih potreba životinja i omogućavanje optimalnog iskorišćavanja lokalnih rasa svinja.

Ključne reči: svinja, porast, prirast, lokalna rasa

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SELECTION OPPORTUNITIES AND MAINTAINING GENETIC DIVERSITY IN LOCAL PIG BREEDS

Dubravko Škorput, Zoran Luković

University of Zagreb Faculty of Agriculture
Svetošimunska cesta 25 10 000 Zagreb Croatia
Corresponding author: Dubravko Škorput, dskorput@agr.hr
Invited paper

Abstract: Breeding programmes in autochthonous pig populations are usually oriented to conservation goals. Selection for economically important traits in such populations can be beneficial for competitiveness of the breed. Autochthonous pig breeds are often characterized by low genetic diversity, and selection in such population becomes demanding. Availability of data and low costs make best linear unbiased prediction (BLUP) the method of choice for selection in conserved populations. Natural mating is dominant in populations of autochthonous breeds, and the consequence of that is the low level of connectedness which makes the comparison of estimated breeding values across herds unreliable. Managing the level of connectedness is dependent on pedigree data quality. Various methods have been developed in order to optimize genetic gain and the level of inbreeding in the population using optimal contribution selection (OCS) framework. OCS is an optimisation of genetic contributions of selection candidates to the next generation such that expected benefit and risks are balanced. A common way to achieve this goal is to maximise genetic gain at a predefined rate of population inbreeding by restricting selection of candidates that are closely related. Pedigree or marker data can be used for that purpose. By the application of OCS genetic, progress can be achieved, that is almost as great as that with truncation selection based on BLUP breeding. Thus, conservation and selection goals can be optimised by the control of the level of connectedness and application of OCS procedures.

Key words: selection, genetic diversity, breeds, conservation

Introduction

The final goal of conservation of endangered populations of pigs is sustainable breeding, which demands development of tools for achieving this goal (*Barker, 2001*). Breeding programmes for local and autochthonous breeds are often oriented

to conservation goals, without intention for genetic improvement of the populations. However, some breeding programmes for pigs include genetic evaluation of economically important traits, such as growth traits in Iberian pig (Duarte *et al.*, 2012). Selection for genetic improvement in economically important traits is a helpful tool for achieving sustainable production and economically effective breeding. By emphasising desirable traits in such breeds, chance of surviving of the breed can be increased. Traits, such as meat quality, might bring additional value for a breed and including these traits in breeding programmes might be beneficial for the conservation process.

Selection for economically important traits, achieving genetic improvement and maintaining genetic diversity in the population seem to be opposing breeding goals, and finding optimal level of genetic improvement and loss of genetic variability becomes challenge for breeders. This is especially important in the breeds of livestock under conservation, which have higher probability for loss of genetic variability and appearance of inbreeding depression, as a consequence of loss of different alleles and increased homozygosity. Thus, breeding programmes for conserved breeds, which usually belong to the group of endangered populations should have balanced breeding goals which include genetic improvement and maintaining genetic diversity.

Genetic diversity

Genetic diversity within breeds of livestock has a crucial impact on sustainability of breeds. In pigs, a few multinational companies run world-wide spread breeding programmes, based on several breeds, which led to negligence and genetic erosion of less productive breeds (Nidup and Moran, 2001; FAO, 2007). The coefficient of inbreeding, inbreeding rate, and effective population size are commonly used measures in describing genetic diversity of the population. The coefficient of inbreeding is defined as probability that two gametes at any locus in an individual are identical by descent. Inbreeding rate refers to the rate at which homozygosity in a population increases: the smaller the population, the faster the increase in homozygosity from generation to generation (Falconer and Mackay, 1996). The measures of the genetic diversity in the livestock populations can be based on pedigree information or molecular data, such as microsatellites or single nucleotide polymorphism (SNP) markers. Moreover, combination of both information sources is beneficial when genealogical information is scarce. However, in well-established breeding programmes where sufficient genealogical information exists, the use of pedigree is sufficient for obtaining reliable information about genetic diversity (Alvarez *et al.*, 2008). In the beginning of the conservation process both sources can be used in order to achieve reliable estimation of genetic diversity parameters.

Such an example can be found in the study of Škorput *et al.* (2018), who used information based on pedigree and microsatellite analysis in the in the Banija spotted pig, an autochthonous breed under conservation process. Obtained results from both sources of information corresponded well with each other, indicating that further efforts in conservation of analysed pig breed should be focused on the creating mating schemes with a goal of controlling the rate of inbreeding and further loss of genetic diversity. Lopes *et al.* (2013) analysed genetic diversity in commercial pig population comparing molecular information obtained by the analysis of SNP markers and pedigree information. The advantage of the first method is better capture of the variation of kinship between sibs due to Mendelian sampling information than in the pedigree-based method.

Local and autochthonous breeds of pigs are often characterised by a low genetic diversity. Low genetic diversity and low additive variance in population does not allow effective selection of the pigs for the traits of interest and these are often limiting factors for genetic improvement of the population. Genetic diversity parameters that describe the genetic variability in the population, such as inbreeding rate and effective population size indicate that diversity of such populations is often low. Such examples are Turopolje pig (Druml *et al.*, 2012) and Red Mangalitza pig (Szabó *et al.*, 2016). Croveti *et al.* (2013) found low effective populations sizes for Italian breeds Mora Romagnola and Cinta Sinese. However, contraction of genetic diversity is not only characteristic of local and autochthonous breeds, but also globally spread pig breeds used worldwide, such as Landrace and Large White (Welsh *et al.*, 2010; Škorput *et al.*, 2019).

Selection of pigs in small populations

Beside conservation purpose, breeding programmes in local and autochthonous breeds of pigs should have developmental role, enabling genetic improvement in the population. According to Gandini and Oldenbroek (2007), strategies for moving from conservation to utilization include defining the relevant breeding goal and marketing products to secure profitability. Thus, genetic evaluation for the traits of interest might be helpful in achieving this goal. Pedigree based genetic evaluation using an animal model is still method of choice in many breeding programmes for pigs. Availability of data and lower costs compared with genomic evaluation makes best linear unbiased prediction (BLUP) the method of choice for selection in conserved populations. However, several pitfalls should be considered when applying BLUP in such population.

Connectedness

Connectedness among contemporary groups is one of the major concerns when a selection of animals across populations (herds, regions, countries or breeds) is performed by BLUP (*Kennedy and Trus, 1993*). This is especially important in the populations where natural mating is the dominant way of mating. *Skorput et al. (2018)* discussed the impact of connectedness between management units on the reliability of breeding values obtained by BLUP in Black Slavonian pig using connectedness rating method. Comparable estimated breeding values between management units (e.g. farms) can be achieved only when sufficient level of connectedness between management units exists. Reliable estimation of the level of connectedness is dependent on data and pedigree quality. The best way to improve the level of connectedness is to organize artificial insemination centres. However, the management of small populations of local pig breeds might not justify such investments. Another efficient way of increase the level of connectedness in populations with dominant natural mating might be an establishing of sire referencing schemes, such as in sheep breeding programmes (*Simm et al., 2001*). Furthermore, boar circle schemes could be considered as an effective way for increasing number of genetic links between management units involved in comparison of estimated breeding values (EBV's). Possible pitfalls in such approach are related with health status risk and this demands strong health control and the application of biosecurity measures.

Optimal contribution selection

Bulmer effect (*Bulmer, 1971*) describes the contraction of genetic variability in the population caused by selection. Moreover, using BLUP as the selection method in the population increases probability of choosing individuals from the best performing families, where individuals contribute more to the gene pool compared to those from lower performing families (*Wray and Thompson, 1990; Wooliams et al., 1999*). Thus, an optimal solution that optimises genetic gain and manage the level of inbreeding in the population is required.

Sustained genetic gain is obtained by creating a covariance between the Mendelian sampling terms (a) and long-term genetic contributions (r) of selection candidates (*Wooliams et al., 1999*). This in turn provides the framework for an effective solution for the management of genomic diversity, due to the relationship between r and rate of inbreeding (ΔF). Various methods have been developed in order to optimize genetic gain and the level of inbreeding in the population using this framework, which is known as optimal contribution selection (OCS) (*Howard et al., 2018*). The optimal contribution selection optimises contributions of selection

candidates to the next generation such that expected benefit and risks are balanced (Woolliams *et al.*, 2015). The concept of genetic contributions was discussed by Woolliams *et al.* (1999) and Bijma and Woolliams (1999). A common way to achieve this goal is to maximise genetic gain at a predefined rate of population inbreeding by penalising selection of individuals that are too closely related (Wray and Goddard, 1994; Meuwissen, 1997). Meuwissen (1997) proposed a method that maximizes the response of selection with a predefined rate of inbreeding, where the optimal solution is expressed as genetic contribution of the individuals to the next generation, which is equivalent to the number of offspring per selection candidate:

$$G_{t+1} = c_t' EBV_t$$

where EBV_t is the vector of BLUP estimated breeding values of the candidates for selection in generation t and c_t is the vector of genetic contributions of the selection candidates to generation $t+1$.

Genomic information is now used in many livestock breeding programs (Garrick, 2011). The EBV's for selection candidates can be thus based on pedigree or genomic data. Moreover, in order to set up inbreeding restriction policy, genomic or pedigree-based measures of coancestry can be used. Clark *et al.* (2013) analysed simulated and real data of dairy bulls, incorporating genomic data into OCS. The use of genomic EBV's resulted in increased genetic gain for a given level of inbreeding when optimal contribution selection was used, especially when the population consisted of large full-sib families. However, in local populations of domestic animals, the genealogical information remains the main source of information about genetic diversity measures.

Caballero and Toro (2000) showed that minimisation of coancestry among individuals enables preservation of the genetic diversity, suggesting independent processes of choice of parents and creating mating schemes. However, Fernandez *et al.* (2001) argued the ability of the practical implementation of optimal conservation strategies, which might be affected by the restricting factors, such as number of individuals offspring can contribute. Also, physiological restrictions can make the application of the optimal contribution strategies harder in populations. For this reason, the authors proposed single step method to optimise the management of a conservation programme when restrictions on the mating scheme exist. The single step method looks for optimal contribution of the parents and sets the optimal mating scheme simultaneously.

Several studies have been published using dairy cattle data (*Koenig and Simianer, 2006; Sørensen et al., 2008; Wang et al., 2017*) horse data (*Solé et al., 2013*) and sheep data (*Henden Kjetså, 2016*). The application of the optimal contribution selection on real data in pigs in the literature is scarce. *Howard et al. (2014)* analysed structural changes in the commercial pig population after applying OCS. They showed that the application of OCS in the genomic evaluation of the pigs has the potential to deliver more genetic gain for the same rate of inbreeding. *Gourdine et al. (2012)* used stochastic simulation with 25 different scenarios in local pig population and found that with optimum contribution selection, genetic progress can be achieved that is almost as great as that with truncation selection based on BLUP breeding. However, for an optimal outcome of applied OCS procedures, pedigree and data quality plays an important role (*Sørensen et al., 2008*).

Conclusion

Selection of the pigs in small local and autochthonous breeds for traits of interest is beneficial and effective if all pitfalls in the process are considered. Although maintaining genetic diversity and genetic improvement seem to be oppose breeding goals, available tools, such as optimum contribution selection procedures, might help breeders and organisations to achieve genetic improvement while maintaining genetic diversity in populations. Characteristic of the local pig breeds is dominant natural mating, which does not allow quick spread of genetic material and thus, genetic improvement improvement is slower. Optimal mating design is the crucial step in achieving satisfying level of connectedness for estimation of comparable EBV's and minimisation of coancestry between individuals in order to restrict the inbreeding rate in the population under selection.

Mogućnosti selekcije i održavanje genetske raznolikosti kod lokalnih rasa svinja

Dubravko Škorput, Zoran Luković

Rezime

Programi uzgoja u autohtonim populacijama svinja obično su orijentisani na ciljeve očuvanja. Izbor ekonomski važnih osobina u takvim populacijama može

biti od koristi za konkurentnost rase. Autohtone rase svinja često karakteriše mala genetska raznolikost, a selekcija u takvoj populaciji postaje zahtevna. Dostupnost podataka i niski troškovi čine najbolje linearno nepristrasno predviđanje (BLUP) metodom izbora za selekciju u očuvanoj populaciji. Prirodno parenje je dominantno u populacijama autohtonih rasa, a posledica toga je nizak nivo povezanosti zbog čega je upoređivanje procenjenih odgajivačkih vrednosti u stadima nepouzđano. Upravljanje nivoom povezanosti zavisi od kvaliteta podataka o pedigreu. Različite metode su razvijene kako bi se optimizirao genetski dobitak i nivo inbreedinga u populaciji koristeći okvir optimalnog odabira doprinosa (OCS-optimal contribution selection). OCS je optimizacija genetskog doprinosa selekcionih kandidata narednoj generaciji tako da se očekivana korist i rizici uravnotežuju. Uobičajeni način da se taj cilj postigne je maksimiziranje genetskog dobitka unapred definisanom stopom inbreedinga populacije ograničavanjem izbora kandidata koji su usko povezani. Podaci o pedigreu ili markerima mogu se koristiti u tu svrhu. Primenom OCS genetike može se postići napredak, koji je gotovo podjednako velik kao i selekcija skraćanja zasnovana na BLUP uzgoju. Stoga se ciljevi očuvanja i odabira mogu optimizirati kontrolom nivoa povezanosti i primenom OCS procedura.

Ključne reči: selekcija, genetska raznolikost, rase, očuvanje

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A GENETIC EVALUATION PROGRAM FOR MEAT QUALITY TRAITS IN IBERIAN BOARS FROM DIFFERENT LIVESTOCK ORIGINS

Juan M. García Casco¹, Juan L. Duarte², Carmen Caraballo¹, Miguel A. Fernández¹, Patricia Palma¹, María Muñoz¹

¹ Centro de I+D en Cerdo Ibérico, Dpto. Mejora Genética Animal, INIA, 06300 Zafra (Spain).

² Association of Iberian Pigs Breeders (AECERIBER), 06300 Zafra (Spain)

Corresponding author: Juan M. García Casco, garcia.juan@inia.es

Invited paper

Abstract: A new genetic evaluation initiative targeting Iberian purebred pigs has been launched in Spain. The program is based on the collection of field and slaughterhouse records in pigs fattened in the extensive system known as *cebo de campo*. The sows, which belong to “La Contienda” farm, are inseminated with boars from other herds registered in the Herdbook. Initial and final weights and intermediate average daily gains are taken at field. At the slaughterhouse, carcass composition traits are recorded and tissue samples collected for fat and meat quality analyses and also for future genomic and transcriptomic analyses.

These records are being used to carry out the racial characterization of the Iberian pig population as well as genetic parameter and breeding value estimation. A preliminary association analysis between quality traits and a 32 SNP panel has been performed. The results show significant associations of SNPs mapped in genes such as *MYH3* and *CASP9* with shear force, thawing and cooking losses and Minolta colour parameters.

The continuity of this program will provide a genetic improvement tool for the breeding association and for the population of Iberian pig, as well as will allow experimental designs for genomic and transcriptomic analyses in order to generate knowledge that would be included in the selection programs.

Keywords: Iberian breed, breeding program, meat quality, candidate genes

Introduction

The Iberian pig is the most abundant autochthonous breed in Europe. However, this breed has scarcely been subjected to the pressure of systematic selection.

Therefore, it maintains an enormous potential for improvement in most of the traits with economic interest.

Recently, new initiatives have begun in this regard, but most of them are aimed at the final production of Duroc x Iberian crossbred pigs. There are few specific selection programs focused on the Iberian purebred pig production. The Spanish association of breeders (AECERIBER) develops the Official Program of the Breed with the support of the Ministry of Agriculture (*Silió, 2000*), with an intra-herd genetic evaluation which limits the progress of the scheme. More recently, one of the most important Spanish traditional companies involved in the elaboration of dry-cured products carries out its own program based on records of *montanera* pigs (final period based on the acorn and grass intake from *dehesa* Mediterranean forest) collected both in the field and slaughterhouse with special attention to meat quality traits (*Muñoz et al., 2016*). Therefore, it is important to highlight that the obtained results are mainly limited to their own herd.

Since 2016, a new experience of genetic evaluation is being carried out by four entities (INIA, AECERIBER, COVAP Livestock Cooperative and La Contienda Foundation) based on records collected in field and slaughterhouse from pigs fattened in *cebo de campo* system (conventional diet but with access to an extensive area of land and available pastures for most of the year). In order to establish a maternal base, sows of the “La Contienda” farm (located in Aroche, Huelva) are being inseminated with boars from several herd origins inscribed in the Herdbook (*Duarte et al., 2017*), which is managed by AECERIBER. Genetic evaluation for growth, carcass and quality traits are being performed, some of them included in the Official Selection Program (*García Casco et al., 2014*), and comparisons between boars are being dispatched to the Association.

The final aim of the program is to provide an objective and reliable tool to the breeders in order to select improved males in a closed future, as well as to generate a database with the characterization of the population and a biobank of tissue samples to be used in future genomic and transcriptomic studies.

Material and Methods

The production system in “La Contienda” farm fits perfectly with the category named *cebo de campo*. After a transition stage up to 90-100 kg with 9-10 months of age in semi-extensive conditions and restricted feeding, pigs are transferred to very large fences with available natural resources (spring and autumn pastures, some acorns in winter, etc.) and liquid feeding system with a gradual increase in the daily ration up to commercial slaughter weight (150-160 kg at 12-13 months of age).

A total of 300 litters have been born in seven production batches, so far (May 2016-May 2019) coming from the insemination of 227 sows with 22 boars. Both the mating and birth have been controlled. Five production batches have already finished their fattening period and have been slaughtered. Data and samples from birth to cutting room of 450 castrated slaughter males are available. The following phenotypic traits and tissue samples were collected:

- Weaning and final slaughter weight and average daily gain (ADG) taken every three months.

- Carcass weight, carcass and ham length, backfat thickness at 10th and last rib, pH in loin at 45' and weight of fatless loins and trimmed hams and shoulders.

- Carcass fat samples from the rump, half loin for quality traits analyses and liver, fat and loin samples for RNAseq analyses.

In addition to this, the forthcoming quality traits analyses will be carried out in fat and loin:

Fatty acid profile in backfat, intramuscular fat, protein and moisture percentages, pH and colour parameters (CIELab coordinates for luminosity (L), redness (a), and yellowness (b); color indexes Hue (H) and Chroma (C)) at 24 h and after thawing, several traits related with water-holding capacity (thawing, cooking and centrifuge force losses), cooked loin tenderness (Warner-Bratzler shear force), marbling by image analysis and myoglobin and collagen content.

All the slaughters are being carried out in COVAP Livestock Cooperative sited at Pozoblanco (Córdoba).

Genetic parameters are being calculated using VCE 5 software (Kovac *et al.*, 2008) and breeding values are being obtained by BLUP-Animal model using PEST 4.1 (Groeneveld *et al.*, 1999). In these analyses, carcass traits are adjusted by the slaughter batch (11 levels) and the maternal origin (three levels: origin La Contienda and other two commercial herds) as fixed effects and by carcass or ham weight as covariates. Quality traits were adjusted by the loin weight as covariate.

A preliminary association analysis between records from 183 animals (Warner-Bratzler shear force, thawing and cooking losses, colour at 24 h) and a 32 SNP panel for meat quality traits in Iberian pig that were designed in a previous study (Fernández-Barroso *et al.*, 2017) were performed. This panel consists of SNPs mapped in the following candidate genes for meat quality traits: *CAPN1*, *CAPN3*, *CAPNS1*, *CAPNS2*, *CASP3*, *CASP9*, *CTSB*, *CTSL*, *MYH1*, *MYH3*, *MYOD* and *PRKAG3*. TaqMan OpenArray platform was used for genotyping and a mixed model including the substitution effect of each SNP (0, 1, 2), slaughter batch and maternal origin as fixed effects and the loin weight as covariate was fitted using Qxpack 4.0 program (Pérez-Enciso & Misztal, 2004).

Results and Discussion

Characterization

Tables 1 and 2 contain mean, maximum and minimum, standard deviation (SD) and coefficient of variation (CV) values from some of the usual production traits and the newest quality traits included in the association analyses. The characterization of the breed will be completed when all the loin samples (approximately 950) will be analysed.

Table 1. Population statistics for some production traits

Trait	Mean	SD	CV (%)	Max.	Min.
ADG growth, g/d	420	94	22.4	664	56
ADG fattening, g/d	626	164	26.2	1092	138
Total ADG, g/d	405	70	17.2	562	125
Final live weight, kg	159.4	9.96	2.94	196.5	142
Hams %	16.74	1.04	6.2	20.60	14.30
Shoulders %	11.02	0.70	6.4	13.60	9.40
Loins %	2.59	0.27	10.5	3.40	1.80
Carcass weight, kg	127.9	8.95	7.0	163.6	107.8

ADG: Average daily day

ADG trait shows great variability (CV = 22% in growth period and CV = 26% in fattening period) and a compensatory effect between them. Faster ADG during the growth period are usually reciprocated with slower growths during the fattening one. Consequently, the total daily average gain shows a lower coefficient of variation. In addition, percentages found in the main cuts are similar to those previously reported in Iberian purebred pig (*García Casco et al., 2014*).

Table 2. Population statistics for some quality traits

Trait	Mean	SD	CV	Max.	Min
W-B shear force, cm ³	2.73	0.66	24.2	1.81	4.51
Thawing loss, %	5.90	2.72	46.1	1.05	18.43
Cooking loss, %	17.32	3.30	19.1	7.89	29.87
L ₂₄	38.84	2.59	6.7	32.46	46.37
a ₂₄	9.61	1.24	12.9	6.37	12.96
b ₂₄	5.29	0.88	16.6	3.11	7.80
L _{Tw}	38.15	1.99	5.2	31.90	43.71
a _{Tw}	9.52	1.11	11.7	6.69	13.10
b _{Tw}	4.13	0.60	14.5	2.84	5.85

L₂₄, a₂₄, b₂₄: Minolta colour parameters measured at 24 h

L_{Tw}, a_{Tw}, b_{Tw}: Minolta colour parameters measured after thawing

Quality trait records are usually more variable than productive ones. In this case, thawing loss behaves as a very heterogeneous trait, with a CV equal to 46%. On the other hand, CV values for L colour parameter (both at 24 hour and after thawing) are below 7%.

Genetic parameters

Table 3 shows heritability values for some of the traits. Some of the values are close to zero (thawing loss, shear force and b_{24}) because of the low number of observations and the sparse depth of the genealogy so far. Re-estimation of parameters is done at each new round of genetic evaluation, with an increasing number of data. Some of these heritability values have been calculated in a commercial closed population of Iberian pigs (*Tavero et al., 2018*), but the heritability estimates here we show are the first obtained in the whole population.

Table 3. Heritabilities of some productive and quality traits

Trait	h^2		Trait	h^2
ADG growth, g/d	0.36		Total ADG, g/d	0.16
ADG fattening, g/d	0.42		Slaughter age, d	0.43
Thawing loss, %	0.06		L_{24}	0.15
Cooking loss, %	0.35		a_{24}	0.17
W-B shear force, cm^2	0.01		b_{24}	0.02
Palmitic, %	0.12		Oleic, %	0.27
Stearic, %	0.54		Linoleic, %	0.66

ADG: Average daily gain

L_{24} , a_{24} , b_{24} : Minolta colour parameters measured at 24 h

Association analysis

The preliminary association analysis were performed with 84 (W-B shear force) and 180 records (thaw and cooking losses and colour parameters). Four out of 32 SNPs were fixed and another one had genotyping problems. Two SNPs located in the gene *PRKAG3* showed a MAF (minor allele frequency) value of 0.03. The remaining 25 SNPs showed $MAF > 0.05$, 10 of them with medium frequencies. Finally, 22 SNPs were used in the association analysis since three of them were co-segregating.

A total of nine significant associations were observed in the total of the nine characters (three for shear force, one for thawing loss, two for cooking loss, one for

L parameter at 24 h and two for b parameter after thawing). Table 4 shows the SNP with the greatest effect for each trait. The allelic substitution of C by A of the MYH3_rs330483504 SNP causes a decrease in shear force, which is related to more tender meats. This gene has already been proposed as a candidate gene for other meat quality traits such as colour, firmness and marbling (Luo *et al*, 2012). In addition, the CASP9_rs34618816 SNP is significantly associated with thawing loss, L at 24 h and b after thawing and the CASP9_rs32430761 SNP with cooking loss.

Table 4. Association of most significant SNPs for some quality traits

	SNP	a (SE)	p-value
W-B shear force, cm ²	<i>MYH3_rs330483504</i>	-0.42 (0.12)	1.49x10 ⁻⁰⁴
Thawing loss, %	<i>CASP9_rs34618816</i>	0.92 (0.30)	0.003
Cooking loss, %	<i>CASP9_rs32430761</i>	-0.86 (0.35)	0.001
Color, L ₂₄	<i>CASP9_rs34618816</i>	0.98 (0.31)	0.002
Color, b _{Tw}	<i>CASP9_rs34618816</i>	0.24 (0.07)	0.001

Conclusion

A genetic evaluation program for Iberian purebred pig is presented in this paper, targeted to carcass trait but emphasizing in meat quality traits. Although the program is still in its first steps, its continuity will provide a genetic improvement tool for the breeder association and for the Iberian pig population, as well as will allow experimental designs for genomic and transcriptomic analyses to generate knowledge that it would be added in the future to the breeding schemes.

Program genetske procene osobina kvaliteta mesa iberijskih nerastova različitog porekla

Juan M. García Casco, Juan L. Duarte, Carmen Caraballo, Miguel A. Fernández, Patricia Palma, María Muñoz

Rezime

U Španiji je pokrenuta nova inicijativa za genetsku procenu iberijskih čistokrvnih svinja. Program se zasniva na prikupljanju podataka sa terena i klanica, svinja koje su tovljene u ekstenzivnom sistemu poznatom kao *cebo de campo*. Krmače, koje pripadaju farmi „La Contienda“, osemenjene su semenom nerastova iz drugih stada koji su upisani u matičnu evidenciju/Herdbook. Početne i krajnje mase i srednji prosečni dnevni prirasti su sakupljeni na terenu. U klanici se evidentiraju osobine sastava trupa i uzimaju se uzorci tkiva za analize kvaliteta masti i mesa, kao i za buduće genomske i transkriptomске analize. Ovi podaci se koriste za obavljanje rasne karakterizacije populacije iberijske svinje, kao i za procenu genetskih parametara i odgajivačke vrednosti. Izvršena je preliminarna analiza povezanosti između kvaliteta i 32 SNP panela. Rezultati pokazuju značajnu povezanost SNP-a mapiranih u genima kao što su *MYH3* i *CASP9* sa snagom presecanja, kalom otapanja i kuvanja i Minolta parametrima boje. Kontinuitet ovog programa obezbediće instrument za genetičko unapređenje za odgajivačku asocijaciju i populaciju iberijske svinje, i omogućiti eksperimentalne dizajne za genomske i transkriptomске analize kako bi se generisalo znanje koje bi bilo uključeno u programe selekcije.

Ključne reči: Iberijska rasa, odgajivački program, kvalitet mesa, kandidatski geni

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PROTEIN AND LIPID METABOLISM AND THEIR INTERACTION IN FATTY (IBERIAN) PIGS

Patricia Palma Granados, Isabel Seiquer, Luis Lara, Ana Haro, Rosa Nieto

Department of Physiology and Biochemistry of Animal Nutrition, Estación Experimental del Zaidín, CSIC, Camino del Jueves, s/n, 18100, Granada, Spain

Corresponding author: Rosa Nieto, rosa.nieto@eez.csic.es

Invited paper

Abstract: The precise knowledge of nutritional requirements of pigs demands previous metabolic characterization. Apart from applied nutritional studies, more basic studies of comparative nature undertaken in the Iberian pig with the aim of elucidating some of the metabolic peculiarities of this fatty pig breed have been developed. These studies and some of their main results and implications are described along this review. This later aspect shows in a way how protein and lipid metabolism can be interrelated, particularly at muscle level. We will deal with amino acid profiles of body proteins, nutritional strategies to increase intramuscular fat and other aspects involving animal welfare such as immunocastration, which deserve attention in this fatty pig breed.

Key words: protein, amino acid, fatty acids, intramuscular fat, Iberian pig

The Iberian pig

Pig production in Spain is the more relevant livestock sector, accounting for nearly 40% of total livestock production in the country. Taken globally, the EU is the second largest pork meat producer after China. Spain is the fourth producing country (after China, USA, and Germany), while, at European level, regarding production Spain is in second place -after Germany- with approximately 18% of total tons produced, and the first in census, with more than 19% of total census (app. 30 million pigs, MAPA, 2019). Within this context, we find the Iberian pig coexisting with contemporary pig breeds. This is an autochthonous breed derived from ancestral domestic pig populations of the Iberian Peninsula, for centuries widely spread all over this territory (*López-Bote, 1998*). Nowadays it can be found in the Southwest of the Peninsula where the Mediterranean-type forest (*dehesa*) still persists. The Iberian pig was the main porcine breed reared in Spain until the

fifties of the last century. Since then, sanitary challenges, changes in social and feeding habits, as well as the transformation of the *dehesa* territory into field crops, led to a dramatic decline in the Iberian pig population leaving the breed at serious risk of extinction (Nieto *et al.* 2019). The recovery began at the late eighties with the revalorisation of its products, the increasing demand for traditional food products of high organoleptic quality, and the social awareness for preservation of the genetic heritage and the natural habitat associated to this breed (Nieto *et al.* 2019). Currently they represent approximately 10% of total pigs produced in Spain, including purebred and crossbred animals (50% Iberian: purebred Iberian dam \times purebred Duroc sire; and 75% Iberian: purebred Iberian dam \times (purebred Iberian dam \times purebred Duroc sire), and this proportion is maintained over the last years (MAPA, 2019).

Metabolic characterization of the Iberian pig and nutritional strategies

This native porcine breed is a slow-growing, fatty-type pig known for the high quality of its products. Previous studies demonstrated that protein deposition is comparatively lower and fat deposition much higher in Iberian pigs along their productive cycle compared with available data from conventional pigs (Nieto *et al.*, 2002; Barea *et al.*, 2007; García-Valverde *et al.*, 2008, Conde-Aguilera *et al.*, 2011). Other comparative studies suggested that rates of muscle protein synthesis and degradation are greater in Iberian pigs, leading to reduced protein accretion (g/d) and less body protein mass in comparison with conventional pigs. In consequence, energy cost of body protein accretion is higher in the native breed as both processes require considerable energy inputs (Nieto *et al.*, 2012). Along with the higher protein turnover rates, higher relative weights of visceral tissues (Rivera-Ferre *et al.*, 2005) makes the Iberian pig less efficient in the use of dietary protein and energy than leaner breeds. According to this metabolic profile, it has been developed a feeding system adapted to the particular metabolic needs of this native pig breed from weaning to slaughter weight (140-160 kg BW). Protein requirement have been assessed by a direct method analysing animal growth and protein-deposition responses to variable protein intakes. Determination of body protein accretion rates have been accomplished by the comparative slaughter technique. By these procedures, both the pig maximal capacity to accrete protein (P_{max} , g/d) and the marginal efficiency of protein deposition (response in protein deposition to changes in energy intake) were determined. Both variables are known to be influenced by genotype and endocrine status. Therefore, the optimal protein/energy

dietary ratio can differ widely for breeds with dissimilar genetic potential for protein accretion. Results are summarised in *Nieto et al. (2012, 2013 and 2014)*.

In all the cited experiments the *ideal protein concept* (i. e., the perfect balance of essential amino acids needed for maintenance and productive functions, BSAS, 2003; NRC, (2012) was followed for formulation of dietary protein. Amino acid (AA) requirements in pigs could be influenced by many factors like sex, genotype, health status, etc., although it has been widely assumed that these factors do not necessarily affect AA composition of body proteins. In addition, previous studies showed differential effects on growth and body protein retention of lysine deficient diets in Iberian and Landrace gilts under similar experimental conditions (*Rivera-Ferre et al., 2006*). On this respect, a study was designed with the aim of determining the optimum lysine proportion of dietary protein for Iberian piglets by analysing responses in growth, carcass nutrient deposition and plasma metabolites (*Nieto et al., 2015*). Six diets containing increasing Lys concentrations at a constant dietary CP content (165 ± 0.7 g/kg DM) were prepared by adding L-Lys HCl at the expense of corn starch (Lys:CP ratios from 43 to 72 g/kg). Carcass protein deposition measurements and plasma urea concentration led to the assumption that optimum Lys:CP ratio for Iberian growing pigs is 64 (*Nieto et al. 2015*), a value somewhat below the established for conventional piglets. This information is valuable for maximizing lean growth and achieving optimum profitability, particularly during the early growth stage, the most efficient phase in conversion of nutrients to animal tissues (*Kendall et al., 2008*).

In general, the AA composition of body protein is considered constant although some studies indicate that AA content of body proteins may be affected by factors such as protein and energy intake (*Bikker et al., 1994*), body weight (*Mahan and Shields, 1998*) and essential AA supply (*Conde-Aguilera et al. 2014*). Changes in the body AA pattern have been related to alterations in the relative proportions of body proteins (actin, myosin, collagen, etc.) with different AA proportions (*Chung and Baker, 1992*). Additionally, deficiencies in essential AA supply have been associated to changes in muscle AA composition and metabolic properties (*Katsumata et al., 2008; Conde-Aguilera et al., 2016*). Pigs with marked differences in body composition and protein deposition rates -like fatty and lean pig genotypes- may differ in AA composition of body proteins. To investigate this issue we tested the hypothesis that if under similar nutritional and physiological conditions pigs from two genotypes, with clear differences in potential for lean tissue deposition (Iberian and Landrace \times Large-White pigs), will have different AA profile of body protein, and will respond differently in terms of AA body pattern to cope with a restricted lysine supply. With this purpose, the AA composition of carcass and muscles of both pig genotypes was assessed in young pigs fed similar amounts of a lysine-adequate or a lysine-deficient diet, both of

similar total protein and ME contents (*Palma-Granados et al., 2017*). Differences in carcass concentration were found for some indispensable AA (isoleucine, valine, and phenylalanine concentrations were higher in carcass protein of Iberian pigs, Figure 1), with higher ratios to lysine observed for Iberian pigs. These findings could indicate relatively higher requirements of these AA for Iberian growing pigs. On the other hand, fatty and lean pigs responded in general terms in a similar way to a deficient lysine supply, modifying the growth rate and modulating the protein composition of specific carcass components, a fact possibly related with their different body functions.

In another set of studies (*Barea et al., 2011*) we investigated possible relationships among differences in nutrient digestibility and nutrient utilization in Iberian and lean-type pigs (at growing and fattening stages, 50 and 115 kg BW) with differences in proportional weight of viscera and micro-structural aspects of the small intestine. It was concluded that these parameters would have minor consequences in nutrient absorption, so that performance differences observed among breeds would mainly be caused by differences in utilization of N absorbed and other digested energy-yielding nutrients.

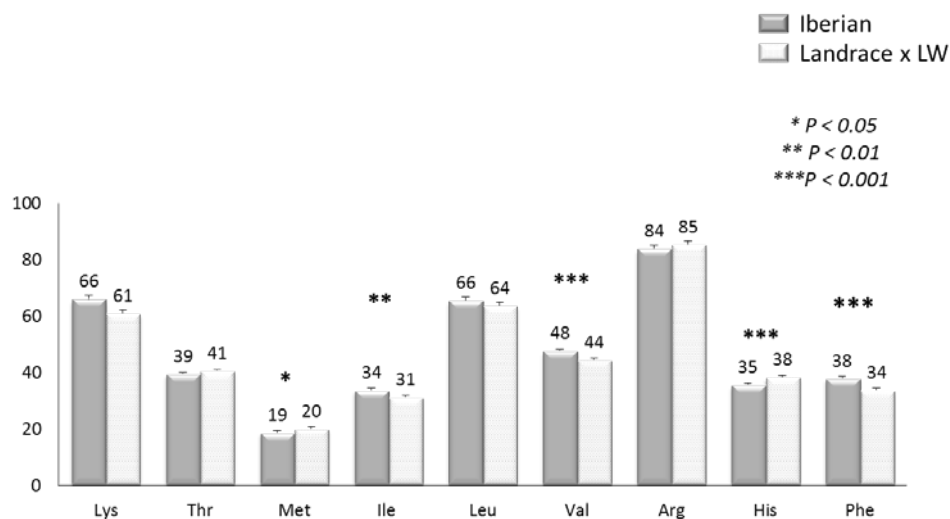


Figure 1. Essential amino acid composition of carcass (g/kg carcass protein) of fatty and lean pigs.

The lipid content and fatty acid (FA) profile in pig tissues are influenced by genotype and nutrient supply, with implications in meat quality. Selection for reduced back-fat thickness in pigs has resulted, at similar dietary FA supply, in lower total saturated (SFA) and monounsaturated (MUFA) and higher polyunsaturated FA content (PUFA) (Scott *et al.*, 1981; Wood *et al.*, 2008). Further comparative studies with the same breed models focused on genotype effects on fatty acid profile of subcutaneous fat (Barea *et al.*, 2013). An experiment was performed to test the effects of pig genotype on evolution of back-fat FA profile throughout the growing and finishing stage using Iberian and Landrace×Large-White barrows under the same nutritional and management conditions. It was confirmed the known influence of genotype on body FA profile and pattern of FA unsaturation during growth, with high proportions of MUFA, particularly of C18:1 n-9, in IB pigs. The results underline the influence of pig genetic variation on *de novo* lipid synthesis and pattern of FA unsaturation. Furthermore, the *de novo* lipid synthesis and pattern of FA unsaturation could be an important cause of variation in the overall efficiency of energy utilization among breeds (Barea *et al.*, 2013).

Overall, the research on this area regarding the Iberian pig fully support the concept of using dietary regimes of protein concentration adjusted to the particular needs of the growth period under consideration (considerable lower in protein for most of growth phases than in conventional pigs) and indicate the pertinence of investigating further possible differences of essential amino acid pattern of dietary protein, more adapted to the metabolic characteristics of the Iberian pig.

Meat quality aspects in comparative studies

The nutritional composition of meat is strongly involved in meat quality characteristics and may be affected by many factors, such as genotype, age, sex, production system and nutritional regime. Meat and meat products of Iberian pigs are highly appreciated by consumers and aspects like amount and composition of intramuscular fat (IMF), and concentration of heme pigments have been highlighted among factors influencing the quality of Iberian meat (Ventanas *et al.*, 2006). The influence of the lipid fraction in the quality of fresh meat has been also well established by other authors (Cameron *et al.*, 2000; Salvatori *et al.*, 2008; Straadt *et al.*, 2013). Colour is also an important characteristic of meat, and attention has been paid to the influence on this parameter of many factors, such as genotype (Lebret *et al.*, 2011), crossbreeding (Carrapiso and García, 2005; Ramirez and Cava, 2007; Fuentes *et al.*, 2014), or production system (Tejerina *et al.* 2012). Heme pigment concentration and IMF have been related with meat colour in several studies (Ventanas *et al.*, 2006; Gonzalez *et al.*, 2012), although their relationship with mineral composition has received scarce attention. However,

mineral content is essential concerning quality and nutritional traits of meat, since meat is an excellent source of minerals such as iron, copper, zinc and phosphorus, and plays an important role in the prevention of zinc and iron deficiency (*Pereira and Vicente, 2013*).

As part of wider project, a study was undertaken to evaluate the nutritional composition of muscles from Iberian and lean pigs and the possible relationship among chemical composition and instrumental colour. Muscles (*longissimus thoracis* and *biceps femoris*) of Iberian and lean pigs (Landrace × Large White, LLW) were compared at two growing stages (50 and 115 kg BW), under identical nutritional and management conditions (*Palma-Granados et al., 2018a*). Nutrient composition, including macro and micro mineral content, FA profile, and instrumental colour of muscles were examined. Although differences were found regarding muscle type and slaughter weight, IB pig muscles contained higher IMF concentration and MUFA proportions, and also higher iron and zinc, and were redder (a^* value) and with less colour saturation (hue angle), than those from LLW animals. Interestingly, significant relationships between colour coordinates and mineral content and lipid profile of muscles were found. Redness was mainly linked to iron, zinc and MUFA levels, whereas yellowness was positively related with copper, PUFA and linoleic acid level (*Palma-Granados et al., 2018a*).

Intramuscular fat

A major factor affecting meat organoleptic characteristics is the amount and composition of IMF (*Wood et al., 2008*), and positive relationships have been established between acceptability or tenderness of pork and IMF content (*Font-i-Furnols et al., 2012*). Different nutritional strategies have been proposed to increase IMF in pigs, as reducing dietary crude protein levels (*Doran et al., 2006*) or reducing dietary lysine (*Katsumata et al., 2005; 2011*). Increase in IMF has also been also reported when decreasing dietary lysine while crude protein was maintained constant (*Tous et al., 2014*). Nevertheless, the effect on IMF seems to be related to dietary lysine shortage. *Kobayashi et al. (2012)* found that decreasing other essential AA as threonine had no significant effect on IMF concentration. On this regard, it has been reported that reduced lysine supply was effective in increasing IMF content in *longissimus* muscle of lean pigs but not in fatty Alentejano pigs (*Madeira et al., 2013*). However, we recently demonstrated that reduced lysine supply, maintaining constant crude protein levels, could be an effective strategy to increase IMF in muscles of both fatty (Iberian) and lean piglets (*Palma-Granados et al., 2017*) maintained in similar nutritional and management conditions. Changes in other biochemical muscle parameters such as increased oleic acid concentration of IMF, and increased proportion of oxidative muscle

fibers in pigs fed a lysine deficient diet were also observed, in agreement with other authors (*Katsumata et al., 2008*).

Besides the effects on IMF, the knowledge of possible effects of lysine deficiency on growth and development of other fat depots deserves attention. *Katsumata et al. (2005)* did not detect any influence of lysine deficiency on subcutaneous backfat-adipose tissue depth, carcass weight, and *longissimus* area in crossbred lean gilts; whereas *Tous et al. (2014)* found no effect of dietary lysine deficiency on backfat proportion or thickness of Landrace \times Duroc finishing barrows. In contrast, we found increased subcutaneous backfat proportions in young Iberian and lean pigs fed a lysine deficient diet (*Palma-Granados et al., 2017*). To unravel potential mechanisms involved in fat deposition promoted by dietary lysine deficiency, particularly IMF, and differential responses between fatty and lean pigs, carcass traits and lipogenic enzyme activities and gene expression levels in muscles and adipose tissue were further investigated. Activity and gene expression of lipogenic enzymes (fatty acid synthase, malic enzyme, and glucose-6-phosphate dehydrogenase) were greater in Iberian than in LDW pigs, particularly in adipose tissue. Regarding the effect of lysine deficiency, in backfat there was no effect of lysine deficiency on activity and gene expression of lipogenic enzymes, nor in gene expression levels of upstream regulators of lipogenesis and adipogenesis, therefore other mechanism besides *de novo* FA and triglycerides synthesis might have been involved in the increase in backfat under these conditions, as increased deposition of dietary FA, or reduced lipolysis rates such (*Palma-Granados et al., 2019*). However, in *longissimus* muscle, the activity of lipogenic enzymes increased with lysine deficiency in both genotypes, and an upregulation of gene expression of lipogenic enzymes was specifically observed in Iberian pigs. Rather similar effects for *biceps femoris* muscle were observed. In both fatty and lean pigs the effect of lysine deficiency on lipid metabolism was tissue-specific, with an activation of lipogenesis in muscles but no apparent stimulation in backfat adipose tissue (*Palma-Granados et al., 2019*). These studies underline that suitable feeding protocols, including lysine-deficient diets, could be designed for each pig type in order to increase intramuscular lipids without penalizing the growth of lean carcass components.

Future perspectives

Although there are many research areas that need to be boosted to improve productivity of Iberian and other fatty pig breeds we are going to mention some relevant aspects in which our research group has been recently involved.

There is an important issue related to animal welfare that concerns the surgical castration of male pigs to avoid boar taint in fresh and cured products. Efforts are being made in this field promoting alternative strategies such as the use of entire males (not viable in the case of native breeds) or immunocastration, the active immunization against gonadotropin-releasing factor. Immunocastration has been described in conventional pigs as an effective strategy to prevent sexual development and the characteristic odor of meat and meat products (*Batorek et al., 2012*), and has great potential in pigs slaughtered at high weights such as native breeds (*Candek-Potokar et al., 2017*). In extensive production, it can be an effective and simple alternative to gonadectomy practiced in Iberian females to avoid the appearance of unwanted estrus or gestation (*Martínez-Macipe et al., 2016*).

However, since the effect of the vaccine is temporal, the long production cycles of the Iberian and other Mediterranean native breeds make necessary to develop vaccination protocols adapted to these production systems (*Seiquer and Nieto, 2018*). Within the TREASURE project, the adaptation of immunocastration protocols to the Iberian and other breeds has been investigated, along with possible effects on the productive parameters and the quality of the products (*Hernández-García et al., 2018*). Studies in our group underline the increased lean growth capacity of male pigs before effective immunocastration (*Palma-Granados et al., 2018b*), although some detrimental effects on meat quality have been detected in premonter male pigs (*Seiquer et al., 2019*). Undoubtedly, further research in this area is needed.

The research program developed on Iberian pig nutrition fully supports the concept of using dietary regimes of dietary protein adjusted to the particular needs of the growth period under consideration as protein requirements suffer a dynamic change along the productive life of pigs. Several advantages are associated to this practice: environmental benefits derived from a more efficient use of protein and the subsequent reduction in nitrogenous wastes, and also the economic benefit derived from protein reduction in feeds, as usually protein is one of the most expensive feed ingredients.

Concerning the field of amino acid requirement in Iberian pig nutrition, a refinement of diet formulation would be desirable in the next years in light of the recent findings regarding potential differences in indispensable amino acid composition in lean tissues of Iberian pigs. Further research in this area is warranted to increase sustainability and profitability of Iberian pig production.

Specific nutritional strategies should be developed for finishing pigs to increase intramuscular fat with expected positive consequences on meat quality. Among this, lysine restriction seems to be effective in fatty and lean pigs. The biochemical mechanisms that contribute to the increase of fat in these conditions seem to differ

among different fat depots and appropriate nutritional strategies should be designed to avoid decrease of growth of lean carcass components. For each pig type, tailored diets -including specific lysine restriction- need to be further developed to schedule duration and specific starting time of the treatment during the fattening period.

Metabolizam proteina i lipida i njihova interakcija kod masnih (iberijskih) svinja

Patricia Palma Granados, Isabel Seiquer, Luis Lara, Ana Haro, Rosa Nieto

Rezime

Precizno poznavanje nutritivnih potreba svinja zahteva prethodnu metaboličku karakterizaciju. Osim primenjenih nutricionističkih studija, razvijene su i osnovne studije uporedne prirode koje se rade na iberijskoj svinji sa ciljem da se razjasne neke metaboličke osobine ove rase masnih svinja. Ova istraživanja i neki od njihovih glavnih rezultata i implikacija su opisani uz ovaj pregled. Ovaj kasniji aspekt pokazuje način kako metabolizam proteina i lipida može biti međusobno povezan, posebno na nivou mišića. Bavićemo se aminokiselinskim profilima telesnih proteina, prehrambenim strategijama za povećanje intramuskularne masti i drugim aspektima koji uključuju dobrobit životinja kao što je imunokastracija, koji zaslužuju pažnju kod ove rase masnih svinja.

Ključne reči: protein, aminokiseline, masne kiseline, intramuskularna masnoća, Iberijska svinja

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HOW NUTRITION MAY INFLUENCE CANINE BEHAVIOR AND COGNITIVE ABILITIES

Giacomo Biagi, Monica Grandi, Carlo Pinna, Carla Giuditta Vecchiato

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

Abstract: Diet can influence people's cognitive functions but, at present, little is known about the role that nutrition can play on the behavior of dogs and cats. Among amino acids, higher tryptophan dietary levels have been linked with reduced anxiety and aggression in different animal species, including dogs. Because tryptophan is in competition with other amino acids when it has to cross the blood-brain barrier in order to be converted to serotonin, the tryptophan to protein ratio may also affect animal behaviour. Interestingly, it has been seen that tryptophan metabolism can also be affected by intestinal microbiota. Alpha-casozepine is a hydrolyzate obtained from bovine milk casein that appears to have anxiolytic properties both in dogs and cats. With regard to lipids, it has been observed that a diet deficient in omega-3 fatty acids may induce aggressive behaviors and a lower resistance to stress in the rat. Carbohydrates may influence behavior of dogs and cats as they can have an effect on their sense of satiety; in particular, diets rich in fermentable fiber may increase the periods of inactivity of dogs for the sense of satiety induced by fiber fermentation. Finally, it has to be remembered that the aging process of companion animals is often associated with a decline in their cognitive function and behavioral changes. In this regard, there is some evidence that supplementing the diet with antioxidants and medium chain fatty acids may improve the cognitive function of elderly dogs.

Key words: Amino acids, Antioxidants, Behavior, Dogs, Fatty acids,

Introduction

In human medicine, the influence that the diet can have on people's cognitive functions (*Stevenson et al., 2014; Hardman et al., 2016*) and on some psychiatric diseases (*Marí-Bauset et al., 2014*) has been the subject of several studies. On the other hand, the number of studies aimed at investigating the role that nutrition can play on the behavior of the dog is quite low and any study concerning the feline species is absent.

There is no doubt that the process of domestication of the dog, starting from the wolf, has significantly changed the behavior of this animal, making it suitable for coexistence with humans. Nevertheless, even today, dog behavioral disorders are one of the main causes of abandonment by the owner and, sometimes, of serious episodes of aggression against people and other animals.

This text aims to analyze the existing scientific literature about the role of nutrition on the behavior and cognitive functions of the dog.

Protein and amino acids

The functions performed by the amino acids that an animal derives from the digestion of dietary proteins are manifold. Among these, in particular, we recall the synthesis of some neurotransmitters, such as serotonin, catecholamine, acetylcholine and histamine, starting, respectively, from tryptophan, tyrosine, choline and histidine (*Young, 1996*). Since the behavior of an animal is influenced by these neurotransmitters it can be thought that any changes in the diet of precursors such as tryptophan and tyrosine can have an effect on behavior.

Among the amino acids precursors of neurotransmitters, tryptophan is certainly the most studied. A diet enriched with tryptophan seems to reduce anxiety in rats (*Orosco et al., 2004*) and aggression in various animal species, including rats (*Kantak et al., 1980*) and monkeys (*Chamberlain et al., 1987*). Furthermore, it appears that tryptophan plays a role in an animal's ability to respond to stress, as observed by *Koopmans et al. (2005)* in the pig. In a 1984 study, *Branchey et al. (1984)* found lower plasma tryptophan levels in human patients suffering from depression than in healthy individuals.

In order for tryptophan to be converted to serotonin, it must cross the blood-brain barrier. The transport through this barrier takes place by a carrier that also carries the other so-called large neutral amino acids (LNAA) that include tyrosine, phenylalanine, leucine, isoleucine and valine, with which the tryptophan is therefore in competition. It follows that the concentrations of tryptophan in the CNS can be increased both by increasing the presence of this amino acid in the diet and by reducing that of the other LNAAs. Since tryptophan is found in food proteins in rather low quantities compared to LNAAs, in general, a diet rich in proteins determines a reduction in the encephalic concentrations of tryptophan (*Leathwood, 1987*).

Tyrosine is the precursor of some catecholamines, including dopamine, adrenaline and noradrenaline. As already mentioned, tyrosine also competes with the other LNAAs for the passage of the blood-brain barrier. Generally, since tyrosine is present in high concentrations in proteins, a high protein diet will determine an increase in tyrosine and a decrease in tryptophan at the CNS level.

The catecholamines that are produced starting from tyrosine seem to play an important role in the animal's response to stress: in fact, in the presence of stressful factors, the consumption of norepinephrine increases considerably and a diet rich in tyrosine can serve to restore its right concentrations, improving the animal's ability to adapt to the cause of stress (*Yeghiayan et al., 2001*).

In the dog, *De Napoli et al. (2000)* observed that the administration of a low protein diet (19% of the diet) supplemented with tryptophan (1.45 g/kg of diet) could reduce the aggressive territorial behaviours of the dogs involved in the study. Conversely, aggressive behaviours increased in dogs fed a high protein diet (31% of the diet) not supplemented with tryptophan. In a more recent study (*Bosch et al., 2009a*), the administration of a diet supplemented with tryptophan (at levels 2.6 times higher than in the control diet) to dogs suffering from anxiety problems did not produce any effect on their behaviour (based on assessments made by the owners) and salivary cortisol levels.

In human medicine it is known that only about 5% of the tryptophan is converted to serotonin, while 95% takes the path of chinurenine, a molecule that inhibits the passage of tryptophan through the blood-brain barrier and that has been seen to have anxiogenic effects in mice (*Marazziti et al., 2013*). Furthermore, tryptophan metabolism can be affected by intestinal microbiota. The bacteria, in fact, degrade part of the diet's tryptophan with formation of indoleacetic acid (IAA) and 3-indolepropionic acid (IPA) while an enzyme present in the intestine, indolamine 2,3-dioxygenase (IDO), transforms tryptophan to chinurenine which will then be transformed by bacteria into kynurenic acid (*Puurunen et al., 2016*). In this regard, it appears that the plasma concentrations of IPA and IAA are inversely correlated with ADHA (hypersensitivity syndrome and hyperactivity) symptoms observed in German shepherds suffering from this problem (*Puurunen et al., 2016*). Conversely, kynurenic acid concentrations would be positively correlated with ADHA events. The interesting aspect is that both IPA and IAA are produced in the intestine by specific clostridial strains and therefore the composition of the microbiota could influence the concentrations of these metabolites and, consequently, the behaviour of the dog.

Alpha-casozepine is a hydrolyzate obtained from bovine milk casein that appears to have anxiolytic properties, as observed both in dogs (*Beata et al., 2007a*) and cats (*Beata et al., 2007b*). In 2010, *Palestrini et al. (2010)* observed that the administration to dogs with anxiety problems of a commercial diet containing alpha-casozepine seemed to improve some behavioral aspects of the dogs. In a subsequent study (*Kato et al., 2012*) conducted on dogs with anxiety problems, the use of a diet enriched with alpha-casozepine (1.35 g/kg of diet) and tryptophan (3.04 g/kg vs 2.83 g/kg of the control diet) reduced some stress parameters in dogs. In this regard, the experimental diet contained more proteins

than the control one (27% against 24%). Similarly, in another study (*Landsberg et al., 2017*), the use of a commercial diet enriched with alpha-casozepine and tryptophan reduced the manifestations attributable to anxiety in the cats involved. In a recent study conducted by *Landsberg et al. (2015)*, the use of a fish hydrolyzate reduced the manifestations of stress (behavioural symptoms and serum levels of cortisol) in Beagle dogs exposed to noises reproducing a storm.

Lipids

As far as lipids are concerned, in the literature there is evidence that an omega-3 fatty acid deficiency (especially DHA) negatively influences CNS development and the cognitive abilities of people and animals (*McCann and Ames, 2005*). However, it has also been observed that a diet deficient in omega-3 fatty acids induces aggressive behaviors (*De Mar et al., 2006*) and a lower resistance to stress (*Takeuchi et al., 2003*) in the rat. A possible explanation for these effects is related to the fact that omega-3 fatty acids influence the metabolism and functionality of dopamine and serotonin (*Bosch et al., 2007*). Unfortunately, to date, there are no specific studies conducted with dogs on the effects that omega-3 fatty acids can have on their cognitive function and behavior.

Carbohydrates

The term carbohydrates refers to both digestible, mainly starch and sugar, and non-digestible carbohydrates, namely dietary fiber. In both cases these nutrients are not essential, even more so in carnivorous animals, but which can still have an effect on animal behavior.

The role of fiber in increasing an animal's sense of satiety and reducing its aggressive behavior has been well documented. For example, the addition to the diet of pregnant sows of large amounts of fiber-rich foods such as wheat bran, soybean hulls and beet pulp makes animals more peaceful and less aggressive (*Bergeron et al., 2000*). In the horse, *Redbo et al. (1998)* observed an inverse correlation between the presence of coarse forages in the diet and the presence of stereotyped behaviors in animals. Despite these evidences, it has not yet been fully clarified how fiber contributes to an individual's sense of satiety. One aspect is presumably linked to the ability of the fiber to stretch the stomach and intestine and to influence gastric emptying. In this regard, it should be remembered that it is the soluble fiber (pectins, gums, mucilage, etc.) that has the greatest capacity to bind water, increasing its volume in the gastrointestinal tract and causing a greater distension of the viscera; moreover, soluble fiber increases the time of gastric

emptying due to the increased viscosity of the stomach contents. Furthermore, the fiber is fermented in the large intestine to volatile fatty acids, which stimulate the production of specific peptides able not only to delay gastric emptying but also to reduce appetite at the CNS level (*Bosch et al., 2007*).

Based on what has been observed in other animal species, it can be assumed that the sensation of hunger influences the behavior of dogs as well. In 1987, Mugford, in a paper aimed at studying the relationship between nutrition and dog behavior, wrote that "when we study a dog's behavior, we should consider what is in his stomach". Some authors (*Jewell and Toll, 1996; Jackson et al., 1997*) have observed how the administration *ad libitum* of high-fiber diets determines in the dog a lower daily energy intake compared to when the animals receive low-fiber diets. However, this effect of fiber on dog satiety was not observed by *Butterwick and Markwell (1997)* in a study conducted with obese dogs subjected to energy restriction. In a more recent study (*Bosch et al., 2009b*), the authors observed that the administration of a diet rich in fermentable fiber increased the periods of inactivity of dogs compared to a diet rich in low fermentable fiber, presumably for a greater sense of satiety induced by fiber fermentation.

The effects of soluble fiber on gastric emptying times were also confirmed in dogs during some studies in which dogs received psyllium fiber and guar gum (*Bueno et al., 1981; Russell and Bass, 1985*). Similarly, although dogs, as carnivorous animals, are characterized by a modest development of the large intestine, the presence of fermentable fiber in their diet leads to the production in the colon of volatile fatty acids, which, once absorbed, could exercise the effects on animal appetite that have been observed in other animal species (*Pouteau et al., 2005*).

Antioxidant molecules, medium chain fatty acids and brain aging

The aging process is often associated with a decline in the animal cognitive function and, sometimes, behavioral changes. Counteracting the decline of cognitive function in elderly subjects, whether they are people or pets, would significantly improve their quality of life. Unfortunately, at present, strategies aimed at avoiding cognitive decline in elderly patients are still at an embryonic stage.

The cerebral aging of the dog appears to be accompanied by the accumulation of oxidation products, with histological aspects similar to those found in human medicine in patients suffering from diseases that lead to dementia (*Cotman et al., 2002*). For this reason, studies aimed at investigating the possibility

of counteracting the decline in the cognitive function of the dog mainly involved the use of antioxidant molecules.

In a study conducted with elderly Beagles, *Milgram et al. (2004)* observed that the administration of a diet supplemented with antioxidants and an environmental enrichment program contributed to improving the cognitive function of the animals. These evidences found confirmation in subsequent studies (*Milgram et al., 2005; Opii et al., 2006*).

Another category of nutrients that has been studied with the aim of limiting the cognitive decline of the elderly dog is represented by medium chain fatty acids (MCT). In a study conducted by Pan et al. (2010), the administration for 8 months of a diet containing 5.5% of MCT to elderly Beagles determined an improvement in the cognitive functions of the dogs (evaluated through specific exercises). Among other things, significantly higher blood concentrations of ketone bodies were found in dogs treated with MCT.

Conclusions

To date, the scientific literature regarding the relationship between nutrition and dog behavior is very scarce. Nevertheless, many evidences gathered in other animal species suggest that even in dogs it is possible to exercise some influence on the behavior of the animal through the diet. In this regard, the protein content of the diet and the concentrations of tryptophan and tyrosine seem to be factors capable of influencing the aggressiveness of the dog. At the same time, concentrations and type of fiber present in the animal's diet could also influence its behavior. Obviously, the feeling of hunger and the sense of satiety can influence an animal's behavior in an important way. Finally, even the composition of the intestinal microbiota could have an influence on the behavior of our animals.

Kako ishrana može uticati na ponašanje i kognitivne sposobnosti pasa

Giacomo Biagi, Monica Grandi, Carlo Pinna, Carla Giuditta Vecchiato

Rezime

Ishrana može uticati na kognitivne funkcije ljudi, ali trenutno se malo zna o ulozi koju ishrana može imati na ponašanje pasa i mačaka. Među aminokiselinama, viši nivoi triptofana u ishrani povezani su sa smanjenom

anksioznošću i agresijom kod različitih životinjskih vrsta, uključujući pse. Pošto je triptofan u konkurenciji sa drugim aminokiselinama kada mora da pređe krvno-moždanu barijeru da bi se pretvorio u serotonin, odnos triptofana i proteina takođe može uticati na ponašanje životinja. Zanimljivo je da na metabolizam triptofana može uticati i crevna mikrobiota. Alfa-kazozepin je hidrolizat dobijen iz kazeina iz goveđeg mleka za koji se čini da ima anksiolitička svojstva i kod pasa i mačaka. Kada je reč o lipidima, primećeno je da ishrana koja ima nedostatak omega-3 masnih kiselina može izazvati agresivno ponašanje i nižu otpornost na stres kod pacova. Ugljeni hidrati mogu uticati na ponašanje pasa i mačaka jer mogu imati uticaja na njihov osećaj sitosti; naročito ishrana bogata fermentirajućim vlaknima može povećati periode neaktivnosti pasa zbog osećaja sitosti izazvane fermentacijom vlakana. Na kraju, treba imati na umu da je proces starenja životinja često povezan sa padom njihove kognitivne funkcije i promenama u ponašanju. S tim u vezi, postoje neki dokazi da dopunjavanje ishrane antioksidansima i masnim kiselinama srednjeg lanca može doprineti kognitivnoj funkciji starijih pasa.

Ključne reči: aminokiseline, antioksidanti, ponašanje, psi, masne kiseline

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ANTIMICROBIAL SUSCEPTIBILITY TESTING OF *STREPTOCOCCUS SUIS* ISOLATES TO COMMON ANTIBIOTICS USED IN PIG FARMS

Aleksandar Stanojković¹, Čedomir Radović¹, Aleksandra Stanojković-Sebić², Marija Gogić¹, Violeta Mandić¹, Jakov Nišavić³, Maja Petričević¹

¹Institute for Animal Husbandry, Belgrade-Zemun, Republic of Serbia

²Institute of Soil Science, Belgrade, Republic of Serbia

³Faculty of Veterinary Medicine, Belgrade, Republic of Serbia

Corresponding author: Aleksandar Stanojković, iza.aleksandar@gmail.com

Invited paper

Abstract: The goal of this study was to determine antimicrobial susceptibility of 34 *Streptococcus suis* strains isolated from healthy and diseased pigs from two pig farms. Disk diffusion was the method used to assess antibacterial susceptibility of *S. suis* according to CLSI (Clinical and Laboratory Standards Institute) and EUCAST standard protocols. Overnight cultures of *S. suis* grown on Colombia 5% blood agar (HIMEDIA, India) were suspended in Todd Hewitt broth (Oxoid Limited, England) and suspension was adjusted to 0.5 McFarland standard. Using sterile swabs suspension was spreaded to Mueller Hinton agar supplemented with 5% defibrinated sheep blood, after which standardized discs containing antimicrobial substances were placed on the agar surface. Plates were incubated at 37 °C in aerobic conditions usually for 18 hours, but no more than 24 hours after which diameter of *S. suis* inhibition zones were measured. Among 34 isolated *S. suis* strains all were resistant to tetracycline (TET), lincomycin (L) and clindamycin (CC). Susceptibility was very low to sulfamethoxazole-trimethoprim (SXT) combination (94.1% resistance), 94.1% of strains were sensitive to penicillin G (P), amoxicillin (AMX), erythromycin (E), enrofloxacin (ENR) and azithromycin (AZM) while sensitivity to cephalosporins of the first (cephalexin-CN), third (cefotaxime – CTX, ceftriaxone - CRO) and fourth (cefepime-FEP) generation, as well as the sensitivity to florfenicol (FFC) and vancomycin (VAN) was absolute (100%). Therefore we can conclude that cephalosporins are a very reasonable choice in treatment of human cases of *S. suis* meningitis but also as a treatment of choice for the *S. suis* pig septicemia.

Key words: *Streptococcus suis*, antimicrobial susceptibility, pig, antibiotics

Introduction

Streptococcus suis is Gram positive bacterium that causes great economic losses to the pork industry. *Streptococcus suis* is the major cause of swine meningitis and septicemia, but also it can be a cause of many other pathological conditions such as arthritis, endocarditis, pneumonia, polyserositis, abortions and abscesses and sudden death (Stanojkovic et al., 2017). Except from being a major domestic swine (*Sus domestica*) pathogen, *S. suis* can be often isolated from variety of other mammalian species and birds. Gottschalk et al. (2010) therefore suggest the existence of complex epidemiological patterns of the infection, since other animal species might also be a source of swine infection (Gottschalk et al., 2010). In Western countries human *S. suis* infections have mainly been considered sporadic but in Asian countries this pathogen is one of the most important cause of meningitis (Arends and Zanen, 1988).

Using coagglutination reaction based on polysaccharide capsules method, thirty-five serotypes (serotype 1 to 34 and serotype 1/2) were previously recognized among of *S. suis* species (Messier et al., 2008). Later, according to DNA based methods serotypes 20, 22, 26, 32, 33, and 34 were reclassified and do not anymore belong to *S. suis* species. Recently, 9 novel serotypes (non-typeable strains) have been identified using DNA sequencing, and at this moment *S. suis* species comprises of 38 serotypes. *Streptococcus suis* serotype 2 is the most frequent serotype causing the disease in both, pigs and humans, but also some other serotypes such as 7, 9, 14, 16, 21 can be involved in the pathologic conditions.

Antimicrobial treatment against infection caused by *S. suis* depends on few criteria's such as bacteria antimicrobial sensitivity, type of infection and the application of the antimicrobials. According to Kataoka et al. (2000), Han et al. (2001) and Marie et al. (2002) high sensitivity to penicillin has been established. However, using MIC method it is shown that that penicillin sensitivity is actually intermediate, while amoxicillin and ampicillin sensitivity is around 90%. So it is suggested that penicillin should be used as a treatment only in the cases of determined susceptibility to this drug.

Antimicrobial resistance of *S. suis* strains to some antibacterial medicines such as tetracycline, clindamycin, erythromycin, kanamycin, neomycin and streptomycin has already been described, while the resistance to trimethoprim-sulfamethoxazole has been very variable. In France, high susceptibility of *S. suis* is shown to be to penicillin, amoxicillin, ceftiofur and gentamicin and very low to lincomycin (38%) and tetracycline (18%) (Marie et al., 2002). In Spain it has been determined (Vela et al., 2005) that over 89% of *S. Suis* strains has been susceptible to penicillin, amoxicillin, ceftiofur, gentamycin, spectinomycin i enrofloxacin while over 87% of strains is resistant to tetracycline, clindamicine and

erythromycin which is attributed to mass use of these medicines in the treatment and disease prevention.

Similar results were obtained in Denmark by *Aarestrup et al. (1998)*. These authors find high resistance to tetracycline and tylosine, high susceptibility to penicillin, ampicillin, ceftiofur and vancomycin, and also variable resistance to macrolide antimicrobials and sulfamethoxazole-trimethoprim combination. *Tian et al. (2004)* six years later find that situation in Denmark has not changed, *S. suis* strains were still susceptible to florfenicol, chloramfenicol, penicillin, ciprofloxacin, sulfamethoxazole and trimethoprim, while high resistance frequency is noted in erythromycin (40.78%) and tetracycline (24.27%).

Wisselink et al. (2006) have investigated *S. suis* susceptibility to different antibacterials currently in use in most of the European countries. In this research all of the strains have been sensitive to enrofloxacin, ceftiofur, cefquinome, florfenicol and penicillin, 1.3% were resistant to gentamicin, 6% were resistant to sulfamethoxazole-trimethoprim combination, while 75.1% were resistant to tetracyclines. Contrary to this, *Tarradas et al. (1994)* in Spain find high resistance to penicillins. Low sensitivity to penicillin (50% of resistant strains) have also been established by *Seol et al. (1996)*, adding that sensitivity to doxycycline was 30%, resistance to amoxicillin, erythromycin and cefalexin was 36.4%, 9.1% and 15% respectively.

In Asia β -lactam antibacterials are still the treatment of choice for *S. suis* infection. Survey in China has showed high resistance to erythromycin, clindamycin, tilmicosine and sulfamethoxazole-trimethoprim (*Zhang et al., 2008*). Ten year survey in Japan which included 689 *S. suis* isolates (*Kataoka et al., 2000*), showed that this bacteria is susceptible to penicillin, cefazolin, ofloxacin and sulfamethoxazole-trimethoprim, while 87% was resistant to tetracyclines, 71.4% was resistant to kanamycin and 29.5% was resistant to streptomycin. In Thailand (*Yongkiettrakul et al., 2019*) it was summarized that *S. suis* strains have high level of antimicrobial susceptibility to vancomycin (96.6%), cefotaxime (93.1%), ceftiofur (94.7%) and florfenicol (92.4%), intermediate level of antibiotic susceptibility to penicillin (33.2%), gentamicin (23.3%), enrofloxacin (21.4%), norfloxacin (27.9%) and sulfamethoxazole-trimethoprim (36.3%) and very low level of antibiotic sensitivity to clindamycin (6.5%), doxycycline (9.2%), tetracycline (5.0%) and tiamulin (2.3%).

Overall, in most of the world countries it is shown that *S. suis* strains are usually resistant in high level to macrolides, lincosamides and tetracycline, variable resistant to aminoglycosides and sulfonamide-trimethoprim combinations, while the resistance to β -lactams, vancomycin, florfenicol, enrofloxacin and ciprofloxacin is usually very low.

Most of the *Streptococcus suis* antimicrobial resistance genes have been identified. Among these, best known and characterized are genes and mechanisms of resistance to macrolides (ermB), tetracyclines resistance (tetM and tetO), penicillins (PBP 1, PBP 2 and PBP 3), fluorohinolones (point mutations) and chloramfenicol (transpozons).

Materials and Methods

Antimicrobial susceptibility was carried out on 34 *Streptococcus suis* strains isolated from healthy and diseased pigs from two pig farms. Method for isolation and identification of *S. suis* isolates was the one described by Stanojkovic *et al.* (2015). Disk diffusion was the method used to assess antibacterial susceptibility of *S. suis* according to CLSI (Clinical and Laboratory Standards Institute) and EUCAST standard protocols. Overnight cultures of *S. suis* grown on Colombia 5% blood agar (HIMEDIA, India) were suspended in Todd Hewitt broth (Oxoid Limited, England) and suspension was adjusted to 0,5 McFarland standard. Using sterile swabs suspension was spreaded to Mueller Hinton agar supplemented with 5% defibrinated sheep blood, after which standardized discs containing antimicrobial substances were placed on the agar surface. Plates were incubated at 37 °C in aerobic conditions usually for 18 hours but no more than 24 hours after which diameter of *S. suis* inhibition zones were measured.

Antibiotic discs used in the survey were the ones often used in pig farms such as penicillin G (P), amoxicillin (AMX), tetracycline (TET), sulfamethoxazole/trimethoprim (SXT), lincomycin (L), erythromycin (E), enrofloxacin (ENR), florfenicol (FFC), cephalixin (CN), but also some used in treatment of human streptococcal infections such as clindamycin (CC), azithromycin (AZM), cefotaxime (CTX), ceftriaxone (CRO), cefepime (FEP) and vancomycin (VAN).

Results and Discussion

Results of antimicrobial susceptibility testing of 34 *Streptococcus suis* isolates are presented in table 1 and table 2 (percentage values).

Table 1. Number of resistant and sensitive isolates of *Streptococcus suis*

<i>Streptococcus suis</i>	Susceptibility	ANTIBACTERIALS														
		P	Amx	Te	Sxt	L	E	Enr	Ffc	Cn	Cc	Azm	Ctx	Cro	Fep	Va
	R	2	2	34	32	34	2	2	0	0	34	2	0	0	0	0
	S	32	32	0	2	0	32	32	34	34	0	32	34	34	34	34

*R –Resistant; S –Sensitive

Table 2. Percentage of resistant and sensitive isolates of *Streptococcus suis*

<i>Streptococcus suis</i>	Susceptibility	ANTIBACTERIALS														
		P	Amx	Te	Sxt	L	E	Enr	Ffc	Cn	Cc	Azm	Ctx	Cro	Fep	Va
	R	5.9	5.9	100	94.1	100	5.9	5.9	0	0	100	5.9	0	0	0	0
	S	94.1	94.1	0	5.9	0	94.1	94.1	100	100	0	94.1	100	100	100	100

*R –Resistant; S –Sensitive

Among 34 isolated *S. suis* strains all were resistant to tetracycline (TET), lincomycin (L) and clindamycin (CC). This corresponds to data provided by Vela et al. (2005) who determined that more than 87% of *S. suis* strains are resistant to tetracycline and clindamycin, as well as the most data collected in European countries (Marie et al., 2002; Aarestrup et al., 1998; Wisselink et al., 2006) that suggest that resistance to tetracyclines, lincomycine and clindamycin is already very high or sometimes total. Same results about low tetracyclines and clindamycin sensitivity were also reported in China by Zhang et al. (2008) in Japan (Kataoka et al., 2000) and Thailand (Yongkiettrakul et al., 2019).

Susceptibility to sulfamethoxazole-trimethoprim (SXT) combination was very low in this research (94.1% resistance). This result was opposite to the result obtained in Denmark (Tian et al., 2004) and most EU countries (Wisselink et al., 2006) which show high sensitivity rate of *S. suis* to this antibacterials. Regarding Asian countries, this result is in compliance to results in China and Thailand but opposite to the one obtained in Japan where sulfamethoxazole-trimethoprim has good activity against *S. suis*.

Explanation for the very high resistance to tetracycline (TET), lincomycin (L) and sulfamethoxazole-trimethoprim (SXT) combination lies in the

fact that these antibacterials were so far the most used drugs in pig farms in „controlled” prevention of different pig diseases. Over the time *S. suis* strains, like many other bacteria became resistant to these antimicrobials.

Like in the most European countries high sensitivity (94.1%) to penicillin G (P), amoxicillin (AMX), erythromycin (E), enrofloxacin (ENR) and azithromycin (AZM) has been found in this research. Unfortunately there are also data that suggest that it might be high or intermediate sometimes (*Tarradas et al., 1994; Yongkiettrakul et al., 2019*). Resistance to erythromycin and azithromycin usually depends on the country or country area, or sometimes on the specificity of the farm in the survey, and it varies from low to intermediate or very high.

Sensitivity to cephalosporins of the first (cephalexin-CN), third (cefotaxime – CTX, ceftriaxone - CRO) and fourth (cefepime-FEP) generation, as well as the sensitivity to florfenicol (FFC) and vancomycin (VAN) was absolute (100%). Although sensitivity to florfenicol is 100% it can be expected that in the near future resistant strains arise because of the more frequent use of this antibacterial in Serbian pig farms. Cases like this are reported in countries where florfenicol is often used to treat pig respiratory infections (*Zhang i sar., 2008*).

High cephalosporin sensitivity is of importance for the human cases of *S. suis* infection. Human cases of *S. suis* infection are usually acquired through close contact with pigs or pig meat, so strains infecting humans usually have same susceptibility to antimicrobials. Reports from human cases of *S. suis* meningitis (*Wangkaew et al., 2006; Lun et al., 2007*) show successful empirical use of third generation cephalosporins in the treatment of the disease caused by this pathogen. Cephalosporins are known to have good penetration into the brain tissue, crossing the blood-brain barrier, have a broad spectrum antimicrobial activity, and like in this research it is shown to have excellent activity against *Streptococcus suis*.

Regarding results in this research we acknowledge that results of antimicrobial susceptibility testing are generally similar to the results other authors find in European countries. Differences can be found not only between countries but sometimes even among pig farms in the same country. Therefore, problem of antimicrobial susceptibility of *Streptococcus suis* in one country must be approached on the systemic micro level, from one to another pig farm.

Conclusion

The results of this study regarding antimicrobial susceptibility testing showed that *Streptococcus suis* strains in Republic of Serbia are most susceptible to cephalosporin antibiotics, but also to florfenicol and vancomycin. High

susceptibility was to penicillin, amoxicillin, erythromycin, enrofloxacin and azithromycin, very low to sulfonamide-trimethoprim combination and all strains of *S. suis* were resistant to tetracycline, lincomycin and clindamycin. Because cephalosporins have a good penetration into the brain tissue and broad spectrum antimicrobial activity, we can conclude that cephalosporins are very reasonable choice in treatment of human cases of *S. suis* meningitis but also as a treatment of choice for the *S. suis* pig septicemia.

Antimikrobna osetljivost izolata *Streptococcus suis* na antibiotike koji se najčešće koriste na farmama svinja

Aleksandar Stanojković, Čedomir Radović, Aleksandra Stanojković-Sebić, Marija Gogić, Violeta Mandić, Jakov Nišavić, Maja Petričević

Rezime

Cilj ovog ispitivanja je bio da se odredi antimikrobna osetljivost 34 soja *Streptococcus suis* izolovanih od bolesnih i zdravih svinja sa dve farme svinja. Disk difuziona metoda je korišćena u ispitivanju osetljivosti prema standardima opisanim od strane CLSI i EUCAST. Kulture *S. suis* dobijene na Kolumbija agaru sa 5% ovčije krvi su supendovane u Todd Hewitt bujon i zamućenje je centrirano na 0,5 jedinica McFarland standarda. Sterilnim štapićem za bris je suspenzija razmazana po Mueller Hinton agaru sa dodatkom 5% defibrinisane ovčije krvi nakon čega su na površinu agara postavljeni diskovi sa antimikrobnim supstancama i agari inkubirani na 37 °C u aerobnim uslovima 18 časova i ne duže od 24 časa. Nakon inkubacije merene su zone inhibicije oko diskova. Svi sojevi *S. suis* su bili otporni na dejstvo tetraciklina, linkomicina i klindamicina, dok je 94,1% bilo otporno na kombinaciju sulfometoksazola i trimetoprima. Nasuprot tome, 94,1% je bilo osetljivo na dejstvo penicilina G, amoksicilina, ertromicina, enrofloksacina i azitromicina, dok su svi sojevi bili osetljivi na dejstvo cefalosporina prve generacije cefaleksina, treće generacije cefotaksima i ceftriaksona i četvrte generacije cefepima, ali i na dejstvo florfenikola i vankomicina. Stoga se može zaključiti da su cefalosporini razuman izbor empirijske terapije meningitisa ljudi koji izaziva *S. suis*, kao i tretman izbora septikemije svinja koju izaziva ova bakterija.

Ključne reči: *Streptococcus suis*, antimikrobna osetljivost, svinje, antibiotici

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THE CHEMICAL COMPOSITION AND QUALITY OF MEAT OF POLISH NATIVE CATTLE BREEDS

Władysław Migdał¹, Bartosz Klusek¹, Łukasz Migdał², Anna Migdał³,
Maria Walczycka¹, Ewelina Węsierska¹, Marzena Zająć¹, Joanna
Tkaczewska¹, Piotr Kulawik¹

¹Department of Animal Product Technology, Faculty of Food Technology, University of Agriculture in Krakow, ul. Balicka 122, 31-149 Kraków, Poland

²Department of Genetics and Animal Breeding

³Institute of Veterinary Science, Faculty of Animal Science, University of Agriculture in Krakow, al. Mickiewicza 24/28, 30-059 Kraków, Poland

Corresponding author: Władysław Migdał, wladyslaw.migdal@urk.edu.pl

Invited paper

Abstract: The domestic (homeland, native) populations - breeds of cattle are these, which are grown up in the certain region and/or country as the of local, primitive breeds. In Poland in above breeds are included 4 breeds: Polish Red cattle (RP), Whiteback cattle (BG), Polish Red-and-White (ZR) and Polish Black-and-White (ZB). Aim of this study was analysis meat from Polish Red and Polish Whiteback young bulls. Meat from Polish Red and Polish Whiteback young bulls were characterized by higher fatness and favorable fatty acids profile. Moreover higher thermal losses were observed. Polish native cattle breeds can be used in production of traditional meat products thanks to good quality of raw meat

Key words: cattle, native breeds, meat, quality

Introduction

The farm animals' accompany human more than 10 thousand years. Over the centuries, man has created about 8 thousand animal breeds. In the FAO database, 7,616 breeds are registered, of which over 85% are so-called local breeds (*Litwińczuk, 2017*). They have given not only food but also helped with farmers' field work, were the components of agri-landscape playing the immanent part of nature, of culture and even ethnography. Nowadays, according to data obtained for 2016 in Poland, the altogether number of registered and preserved with *in situ* programs, is 83 breeds, varieties and lines, where 43 are mammals, 35 domestic birds and 5 bees lines. They are breed in 3259 farms (*Krupiński et al., 2017*).

The native populations breeds are these, which are grown in certain regions and/or country as the local, primitive breeds (*Hoffman, 2010*). According to FAO

DAD-IS (www.fao.org/dad-is) there are 618 European cattle breeds, while 547 of them were classified as local or regional breeds e.g. Asturiana, Morucha, Rubia Gallega, Pajuna, Mirandesa, Arouquesa alentejana, maronesa, mirandesa, Romagnola, Dutch Belted, Lakenvelder, hungarian Grey. In Poland in above breeds are included 4 breeds: Polish Red cattle(RP), White-backed cattle(BG), Polish Red-and-White (ZR) and Polish Black-and-White (ZB). Population of native cattle breeds significantly decrease, therefore in 1999 Lesserpoland Cattle Breeders Association suggested establish rules and financial support for protection of genetics resources of Polish Red cattle. Since 2003 similar programme were launched for Polish Whiteback cattle (*Krupiński et al., 2012*). Polish Red cattle derives from small wild brachycerous (short-horned) cattle, living in the eastern part of Central Europe and in Scandinavia. As of 15 March 2018, 2419 cows tested for milk yield from 259 herds and 577 cows tested for meat performance from 40 herds were qualified for inclusion in the conservation programme (*Majewska, 2019*). At the beginning of the 20th century, White-backed cows formed around 10% of the whole population on the right bank of the Vistula River, and in the Wieprz, Pilica, Narew, Bug and Liwiec River valleys. As of 15 March 2018, 610 cows from 58 herds were qualified for inclusion in the conservation programme (*Majewska, 2019*). Lowland spotted cattle came to Poland in the Middle Ages together with Dutch settlers. By the early 20th century they occupied extensive areas of Poland and were considered a native breed (Black-and-White Lowland). As of 15 March 2018, 1640 cows from 115 herds as well as 370 heifers were qualified for inclusion in the conservation programme (*Majewska, 2019*). Red-and-White cattle has been raised in Western Europe since the 13th century. Until the first half of the 17th century, this cattle was the dominant spotted breed in lowland regions of Europe, but they were driven out by Black-and-White cattle. Red-and-White cattle has been raised on Polish land for more than a century, when they were brought to Poland from Westphalia, Rhineland and East Friesland, first to Lower Silesia and Opole region, and later to Southern Poland. As of 15 March 2018, 3395 cows from 330 herds were qualified for inclusion in the conservation programme (*Majewska 2019; Litwińczuk et al., 2014*) showed that polish native cattle fit extremely good In semi-intensive fed using grass-fed diet and can be characterized by high dressing percentage. Advantages this breeds are good quality raw meat, lower feeding demands, natural resistance for worse environmental conditions and higher resistance against illnesses and stress in comparison to regular industrial breeds. They are outstanding for high reproduction traits and well survival ability in difficult environmental conditions (*Litwińczuk, 2017*). Polish Red cattle and Polish Whiteback cattle are part of polish husbandry heritage. For increasing interest of breeders it is necessary to promote meat and milk products from those breeds. Current results support earlier information about

good meat quality, high mineral content and favorable fatty acids profile (Domaradzki et al., 2016b; Florek et al., 2017; Iwanowska et al., 2010; Litwińczuk et al., 2016). Traditional feeding based on pastures can be characterized by favourable $n-6/n-3$ proportion, and higher content of PUFA (especially $n-3$) (Florek et al., 2017). At a meat outlet, consumers judge the quality of beef by its colour, visible fat content, consistency, and odour (Kolczak, 2008).

Aim of this study was analysis of meat from Polish Red and Polish White back cattle young bulls.

Material and Methods

In scope of the project “The uses and the conservation of farm animal genetic resources under sustainable development” co-financed by the National Centre for Research and Development within the framework of the strategic R&D programme “Environment, agriculture and forestry” – BIOSTRATEG, contract number: BIOSTRATEG2/297267/14/NCBR/2016 were analysed meat of native Polish cattle breeds. Samples used in present study were collected from *musculus longissimus lumborum* - MLL and *musculus semitendinosus* – MST collected from carcasses of 6 young bulls of Polish Red and Polish Whiteback. Bulls were kept in small farms and pasture-raised. In the age of 18 months they were slaughtered in Professional slaughterhouse. Results obtained from Polish native cattle breeds were compared with results obtained from crossbreeds for Polish Holstein-Friesian and Charolaise (PHFxCH) from intensive feeding. Meat samples were analyzed after 7 days chilling

The obtained samples of meat were comminuted and subjected to physico-chemical analyses. The following items were estimated in the raw meat samples:

- water content according to the standard (PN-ISO 1442:2000),
- fat content according to the standard (PN-ISO 1444:2000),
- protein content by Kjeldahl method (PN-75/A-04018),
- total ash content according to the standard (PN-ISO 936:2000),
- total carbohydrates content was calculated assuming that the all total solids and water stand for 100%,
- the measurements of colour of meat and tenderloins samples were obtained in CIELab system (CIE, 2004). Lightness [L*], redness [a*] and

yellowness [b*] of meat were determined using a Konica Minolta CM-600d spectrophotometer,

- meat weight cooking loss. Samples were heated to reach the internal temperature of 75°C in the geometric centre of the sample. The results were computed from the difference between the weight before and after cooking,
- The measurement of shear force was performed with Warner-Bratzler triangle edge knife. From each lot were cut out 5 cylindrical samples (diameter 14mm , height 15mm) and the force needed for rectangular samples cutting was measured. The test blade speed was 2mm/s.

Statistical analysis

All samples were obtained at least in duplicates. All results were analysed with ANOVA and present as means with standard deviation. The calculations were performed with Statistica 6.0 (*StatSoft, 2003*).

Results and Discussion

In Table 1 chemical composition of samples from *musculus longissimus lumborum* and *musculus semitendinosus* collected from young bulls from native polish breed and intensively feed crossbreeds were presented. There were no statistical differences expect for fat content. According to *Florek et al. (2017)* muscles content of chemical components differ and *longissimus dorsi* muscle had the highest content of protein. Level of protein in muscle is between 19% and 23%. Crossbreeds young bulls from intensive feed were characterized by lean meat – statistically lower content of intramuscular fat compared to native polish cattle. According to *Daszkiewicz et al. (2001)* and *Litwińczuk et al. (2012)* optimal content of intamuscular fat in cattle meat is estimated between 2,5 and 4,5 %. Analysed meat of Polish Red and Polish Whiteback young bulls fit this criteria. *Wajda et al. (2014)* reported that is may be hard to achive this values in meat from Holstein cattle. According to *Wichlacz et al. (1998)* and *Trela et al. (2004)* intramuscular fat content on level 1% is a minimal amount for proper sensoric quality of meat. *Nogalski et al. (2013)* reported higher intremuscular content (max 4,71%) in muscle *longissimus dorsi* from crossbreeds of Polish Holstein-Friesian cows with Limousine bulls. *Florek et al. (2017)* reported that *longissimus dorsi* muscle intramuscular fat content is higher compared to *semimembranosus* i

semitendinosus muscles. It must be stated that intramuscular fat content influence on tastiness and meat texture.

Fatty acids profile is another important parameter of meat quality. *Florek et al. (2017)* and *Litwińczuk et al. (2016)* showed that in cattle intramuscular fat monounsaturated fatty acids are most common - MUFA (46%), saturated fatty acids- SFA (44%) and polyunsaturated fatty acids - PUFA – 10%. Most common fatty acids are 14:0, 16:0 i 18:0 and oleic acid (18:1n-9). PUFA:SFA ratio in beef meat is lower and differ between 0.06 to 0,75 (*Florek et al., 2017*).

Ratio n-6:n-3 in beef fat mostly depends on feeding system and differ between 1.7 (foraging) and 24.5 (concentrate feed) (*Florek et al., 2017*). Most common PUFA n-3 in beef meat are α -linoleic acid (18:3n-3) and long-chained: eicosapentaenoic (20:5n-3) and docosapentaenoic acid (22:5n-3). Beef fat is a source of conjugated linoleic acid (CLA) where most common isomer is *cis*-9, *trans*-11, - about 72–90% total level of CLA. This isomer is synthesized by *Butyrivibrio fibrisolvens* bacteria in rumen in process of hydrogenation of linoleic and linolenic acid (*Bartnikowska, 2001*).

According to *Zymon (2014)*, beef from cattle feeding on pastures can be characterized by higher level of linolenic acid and lower content of linoleic and oleic acids compared to animals feed by concentrate feed. Therefore n-6:n-3 ratio in cattle feed on pastures is more favorable (*Florek, 2017*).

Table 1. Chemical composition of *lumborum* part of muscle *m. longissimus dorsi lumborum* (MLL) and *semitendinosus* muscle *m. semitendinosus* (MST) bulls

Chemical component [%]	<i>lumborum</i> part of muscle <i>m. longissimus dorsi lumborum</i> (MLL)			<i>semitendinosus</i> muscle <i>m. semitendinosus</i> (MST)		
	Breed of cattle			Breed of cattle		
	Polish Red (RP)	White-backed (BG)	Cross-breeds PHFxCh	Polish Red (RP)	White-backed (BG)	Cross-breeds PHFxCh
Water	74.51±1.25	75.39±1.52	76.11±1.99	74.33±2.05	74.58±1.01	76.07±1.50
Total solids	25.49±1.28	24.61±1.56	23.89±2.01	25.67±2.08	25.42±0.98	23.93±1.43
Protein	21.97±2.85	21.27±3.07	21.05±2.12	22.11±1.98	21.95±2.12	21.33±1.96
Fat	2.30 ^a ±0.31	2.08 ^b ±0.24	1.57 ^c ±0.19	2.31 ^f ±0.18	2.16 ^e ±0.25	1.28 ^g ±0.11
Ash	1.11±0.05	1.09±0.08	1.15±0.04	1.15±0.1	1.13±0.07	1.19±0.02
Carbohydrates	0.11±0.01	0.17±0.02	0.12±0.01	0.10±0.018	0.18±0.01	0.13±0.009

a, b, c, e, f, g Mean values in lines marked with a different letters differ statistically significantly at $P \leq 0.05$

In Table 2 we presented parameters *m. longissimus dorsi lumborum* (MLL) and *m. semitendinosus* (MST) bulls and thermal losses. Meat of analyzed bulls characterized surprisingly high thermal losses at level of 41,93%. *Litwińczuk et al. (2014)* reported higher thermal losses after 7 days chilling in Simental and Polish Holstein-Friesian young bulls meat (31,6 and 31,8 %) compared to polish native breeds - Polish red, Polish Black-white and Polish Whiteback (28,1 ÷ 28,9 %).

In our study there were differences in colour parameters of young bulls meat. Meat from crossbreeds bulls were darker, with more redness and less yellow compared to polish native breeds. *Litwińczuk et al. (2014)* reported statistically lower concentration of heme pigment in Simental and Polish Holstein-Friesian compared to native breeds. It was evidenced that the heme pigment contents in beef were correlated with the a^* value (at approx. 0.50) (*Florek et al., 2009*).

Table 2. Parameters of lumborum part of muscle *m. longissimus dorsi lumborum* (MLL) and semitendinosus muscle *m. semitendinosus* (MST) bulls

Parameters	<i>lumborum</i> part of muscle <i>m. longissimus dorsi lumborum</i> (MLL)			<i>semitendinosus</i> muscle <i>m. semitendinosus</i> (MST)		
	Breed of cattle			Breed of cattle		
	Polish Red (RP)	White-backed (BG)	Cross-breeds PHFxCh	Polish Red (RP)	White-backed (BG)	Cross-breeds PHFxCh
Thermal losses %	41.93±3.87	39.76±3.86	39.62±2.68	36.66±4.02	39.98±3.66	40,16±3,12
Colour						
L*	37.94 ^a ±3.91	45.74 ^b ±3.03	34.58 ^a ±2.76	44.28 ^r ±3.24	49.10 ^f ±2.79	36,22 ^s ±2,74
a*	11.15 ^a ±1.57	8.03 ^b ±1.10	16.49 ^c ±1.96	7.41 ^r ±1.36	8.88 ^r ±1.02	18,89 ^s ±1,01
b*	10.43 ^a ±1.33	11.07 ^a ±1.21	7.42 ^b ±1.01	13.51 ^r ±1.13	14.95 ^r ±1.14	8,91 ^s ±0,89

^{a, b, c, r, s} Mean values in lines marked with a different letters differ statistically significantly at $P \leq 0.05$

In Table 3 texture and shear force parameters of *m. longissimus dorsi lumborum* (MLL) and *m. semitendinosus* were presented. After cooking meat from native breeds differ in hardness and shear force parameters. Most favourable parameters were found for Polish Whiteback meat, where lowest shear force were found. It may be connected with collagen content or different number and diameter of muscle fibres. This hypothesis must be verified.

Table 3. Texture profile and shear force for of muscle *m. longissimus dorsi lumborum* (MLL) and semitendinosus muscle *m. semitendinosus* (MST) bulls

Breed of cattle	Texture profile					Shear force [N]
	Hardness [N]	Springiness	Cohesiveness	Chewiness [N]	Resilience	
muscle <i>m. longissimus dorsi lumborum</i> (MLL)						
Polish Red (RP)	119,2 ^a ± 11,21	0,457± 0,04	0,481± 0,03	26,18 ^a ± 2,11	0,165± 0,01	22,08 ^a ± 1,91
White-backed (BG)	109,89 ^b ± 9,21	0,563± 0,04	0,47± 0,05	29,27 ^a ± 2,02	0,142± 0,008	19,87 ^a ± 1,76
Cross-breeds PHFxCh	105,26 ^b ± 10,65	0,417± 0,03	0,439± 0,02	19,27 ^b ± 1,84	0,142± 0,009	26,03 ^b ± 2,91
semitendinosus muscle <i>m. semitendinosus</i>						
Polish Red (RP)	114,60± 12,14	0,426± 0,05	0,521± 0,02	36,44 ^b ± 3,41	0,211± 0,01	20,15± 2,01
White-backed (BG)	112,97± 10,11	0,489± 0,06	0,446± 0,03	24,64 ^a ± 2,53	0,147± 0,008	19,87± 2,45
Cross-breeds PHFxCh	129,64± 13,56	0,594± 0,03	0,542± 0,01	45,10 ^c ± 7,04	0,237± 0,009	22,63± 3,41

a, b, c, r, s Mean values in columns marked with a different letters differ statistically significantly at $P \leq 0.05$

Conclusions

Available literature data suggest good quality of meat from native breeds. Domaradzki et al. (2016a) and Litwińczuk et al., (2016) confirmed high content of minerals and favorable fatty acids profile. Moreover, they indicates that meat from animals kept in traditional way on pastures characterized by favorable *n-6/n-3* proportion, higher PUFA content especially *n-3* fatty acids.

Meat from Polish Red and Polish Whiteback were characterized by higher fatness and favorable fatty acids profile compared. However, also higher thermal losses were reported. Meat from polish native breeds seems to be good quality material for production of traditional meat products.

Hemijski sastav i kvalitet mesa poljskih autohtonih rasa goveda

Władysław Migdał, Bartosz Klusek, Łukasz Migdał, Anna Migdał, Maria Walczycka, Ewelina Węsierska, Marzena Zajac, Joanna Tkaczewska, Piotr Kulawik

Rezime

Domaće (autohtone, nativne) populacije - rase goveda su one, koje su odrasle u određenom regionu i/ili zemlji kao lokalne, primitivne rase. U Poljskoj tu se ubrajaju 4 rase: poljska crvena goveda (RP), bela goveda (BG), poljska crveno-bela (ZR) i poljska crno-bela (ZB) goveda. Cilj ove studije bila je analiza mesa junadi poljske crvene i poljske bele rase. Meso poljskih crvenih i poljskih belih junadi odlikovala je veća masnoća i povoljan profil masnih kiselina. Takođe su primećeni veći toplotni gubici. Poljske rase domaćih goveda mogu se koristiti u proizvodnji tradicionalnih mesnih proizvoda zahvaljujući dobrom kvalitetu sirovog mesa.

Ključne reči: goveda, autohtone rase, meso, kvalitet

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A COMPUTERISED SYSTEM FOR PREDICTION OF SLAUGHTERING CHARACTERISTICS OF BEEF CATTLE

Yalçın Bozkurt¹, Tuncay Aydoğan², Cevdet Gökhan Tuzun¹, Cihan Dogan¹

¹ Isparta University of Applied Sciences, Faculty of Agriculture, Department of Animal Science, Isparta, Turkey

² Isparta University of Applied Sciences, Faculty of Technology, Department of Software Engineering, Isparta, Turkey

Corresponding author: Yalçın Bozkurt, yalcinbozkurt@sdu.edu.tr

Invited paper

Abstract: A computer program developed on the basis of both Linear Regression (LR) models and Artificial Neural Networks (ANN) models for prediction of live weight and some carcass characteristics such as carcass weight and yield. This software is connected with a camera system when used in farm or slaughter houses which can be installed on personal computers. The models developed by LR and ANN in previous studies were used for the development of the software application. For testing, the data were collected as independent data from the pre-slaughtered beef cattle regardless of the type of breeds and were used for the validation of the models and for determining the performance of software application. It was observed that the prediction ability of the models were evaluated after having a high prediction levels with $\pm 3\%$ kg error margin for each carcasses. For the validation of the models, the data with regardless of the breeds were collected from the beef cattle brought to a private slaughterhouse. The prediction ability of the models were evaluated after having a high prediction levels with $\pm 3\%$ kg error margin for each carcasses. Then a computerized system was developed not only for slaughter houses but also beef producers to provide information about slaughtering characteristics of beef cattle and improve managerial skills of slaughter houses.

Key words: prediction models, artificial neural networks, linear regression, slaughterhouse

Introduction

The use of electronics in agriculture has gained increasing interest for the last decade, especially beef producers who have computers frequently ask about the availability of software programs to handle their production systems and keeping records. For those who run slaughter houses, it is important to use a computerised system with regard to making better management decisions and speeding up slaughtering process.

In recent years, web-based software programs have become widely used in sectors due to the rapid development of computer and internet technologies and the many facilities provided by hardware and software. In this context, Digital Image Processing (Digital Image Processing) methods have started to find new usage areas (*Cross and Belk, 1994*). Numerical Image Analysis and processing methods which are used in science, computer science, mathematics, physics and statistics can be applied in many fields such as agriculture, forestry, engineering and medicine. Likewise, it is possible to obtain some spatial dimensions such as length, area and angle from the camera images by digital image analysis in computer environment. Therefore, in many stages, such as automatic product identification, classification, data acquisition and interpretation in animal production are needed for development of software applications (*Bozkurt et al., 2017*).

Many research results have shown that the data obtained before slaughter can be predicted by using the ANN method (*Brethour, 1994; Li et al., 1999; Hill et al., 2000; Hatem et al., 2003; Adamczyk et al., 2005*) while previously there have been reports of using linear regression models to predict beef performance and carcass characteristics (*Heinrichs et al., 1992; Enevoldsen and Kristensen, 1997; Fourie et al., 2004; Bozkurt, 2006; Bozkurt et al., 2007, 2008, 2017*).

Therefore, in this study the development of a web-based computer program was aimed to estimate the carcass characteristics of slaughtering beef cattle, such as live weight, carcass weight and yield. For this purpose, the models obtained in the previous studies were used to develop a computerised system for beef producers and slaughter houses.

System Components

The system components consist of three stations, a network for data communication between stations, an internet connection for accessing remote databases, and a computer program developed. Automation system components

include hardware and software components. Hardware items include paddock, live weight scale, carcass weight scale, 3 cameras, Wi-Fi Access Point and computer. All system components are connected with high resolution and web based IP cameras to take side and top images of animals before and after slaughter.

Entry into the system

The system can be installed and run on windows operations. After installation a programme launch icon is created on desktop and with the clicking on the icon, Login window appears with language selection button as shown in Figure 1.



Figure 1. System entry screen

On the Settings tab, a new slaughterhouse and software user registration is defined. Enter the Server Name, Database, Username and Password information for registration and TEST CONNECTION button is pressed (Figure1). If the test fails, Test Connection Error appears on the screen, “Registry Save Error warnings are encountered. If there are no problems, the entry into system is realised.

Receiving live animal and carcass images

With the Live Animal Image Import menu, the image stream is started by connecting to the cameras receiving top and side video images of the live animal on the animal turnstile (shut or cage). If the connection with the camera can not be established, a warning message appears. If a connection is established, a window named Get Live Image command appears on the screen.

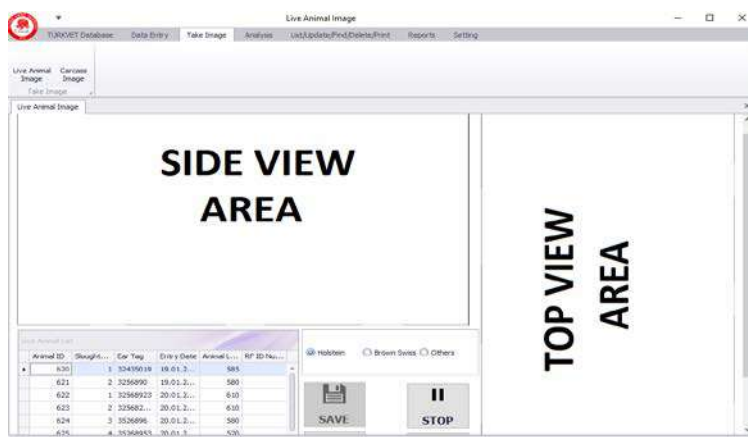


Figure 2. Screen for receiving live animal image

In the Get Live Image window in Figure 2, the live animal on the turnstile has real-time video footage from the top and side. In addition, the animal in the image, Animal ID, Daily Slaughtering No, Ear No, Entry Date and Radio Frequency Identification (RFID) No and optional breed information is included in the Live Animal List, the area where the animal breed can be selected and the START / STOP, SAVE, DISCONNECT CAMERA and CONVERT buttons.

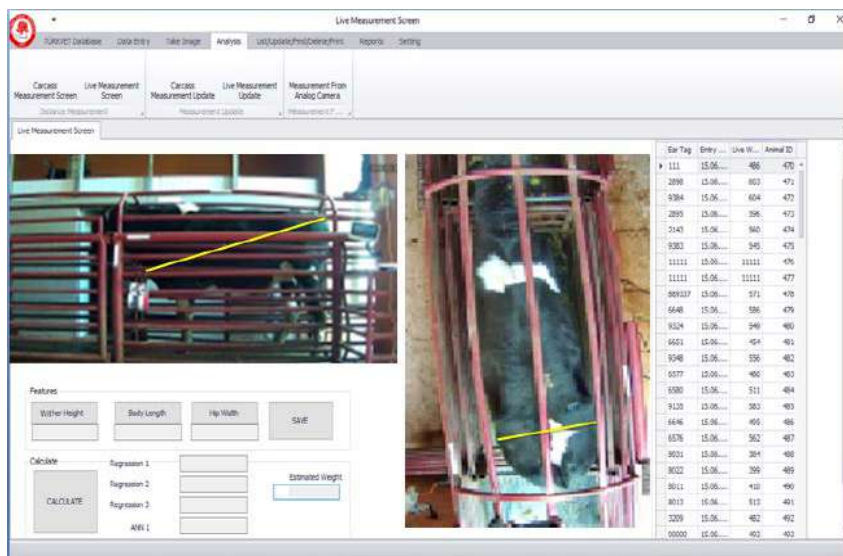


Figure 3. Live Body Measurement Screen

The user first selects the menu at the top of the Live Animal List screen with the animal's information displayed on the screen by pressing the left mouse button, then using the START / STOP buttons to obtain the instant image of the live animal, selects the animal's race and saves the animal's information along with the image format image with the SAVE button. The action OK message, indicating that the registration process is complete, appears on the screen, and the recorded animal information disappears from the Live Animal List. Along with the message displayed on the screen, the second user with mobile computer next to the turnstile will be sent to the screen by sending the live animal from the turnstile to the slaughter and ensuring that the next live animal is put on the turnstile and ready for expose of next images.

The CUT CAMERA CONNECTION button disconnects the image stream from the cameras to the screen when there are no live animals during the turnstile. The image stream can be restarted with the START button. Finally, The CONVERT button enables the Live Animal List to be produced in .pdf file format.

When the Live Measurement menu is selected, the Live Measurement Screen window in Figure.3 is displayed. The side view and top view photo of the selected animals in the Animal List is displayed. The body length is marked as a line with the mouse and then the hip width is taken by pressing the hip width button. The same procedure can be repeated to take the wither height and body depth. When the CALCULATION button is pressed, the weights calculated with the Regression and ANN mathematical models are seen in the Method1 and Method2 fields according to the measured lengths. The live weight is predicted. Pressing the SAVE button saves the data for this animal. The recorded animal information disappears from the list and the next animal is ready for image taking and measuring.

Obtaining carcass images

With the Get Carcass Image menu, the image stream is started by connecting to the camera that receives video from the length of the suspended carcass. If the connection with the camera cannot be established, a warning message appears. If the connection is established, a window called Get Carcass Image in Figure 4 opens.

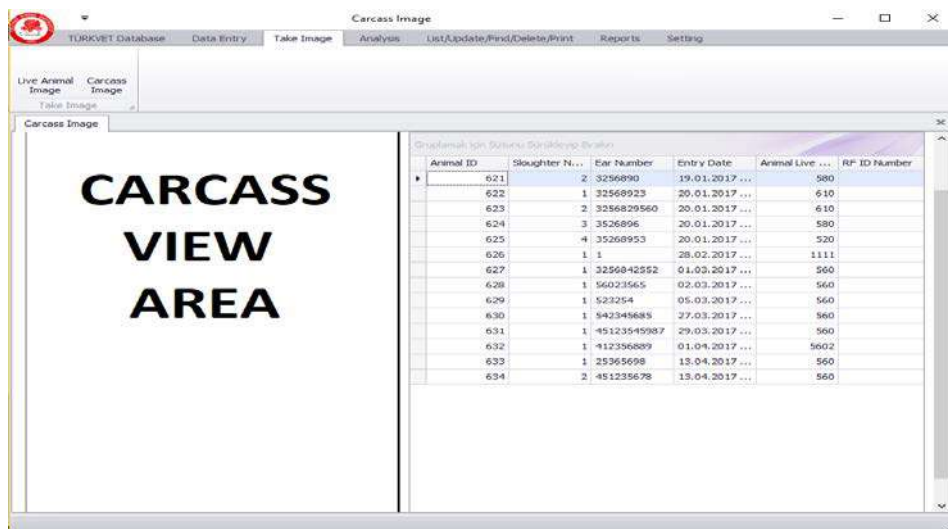


Figure 4. Receiving carcass image screen

In the Get Carcass Image window in Figure 4, a real time video image of the suspended carcass is shown. In addition, the carcass in the image contains the Animal ID, Animal Slaughtering Sequence, Date / Number, Ear No., Date of Entry, Weight, and RFID No information.

The user first selects the line at the top of the list with the information of the incoming animal by pressing the left mouse button, then using the START / STOP buttons to obtain the instant image of the carcass and saves the image format image with the SAVE button. The action OK message, which states that the registration is complete, appears on the screen, and the information of the recorded animal disappears from the Carcass Animal List. With the message on the screen, the person next to the hanger removes the carcass hanger from the exposure area and moves the next carcass hanger to the exposure area. The CUT CAMERA CONNECTION button interrupts the display flow from the cameras to the screen when there is no suspended carcass. The image stream can be restarted with the START button.

The CONVERT button enables the Carcass Animal List to be produced in .pdf format.

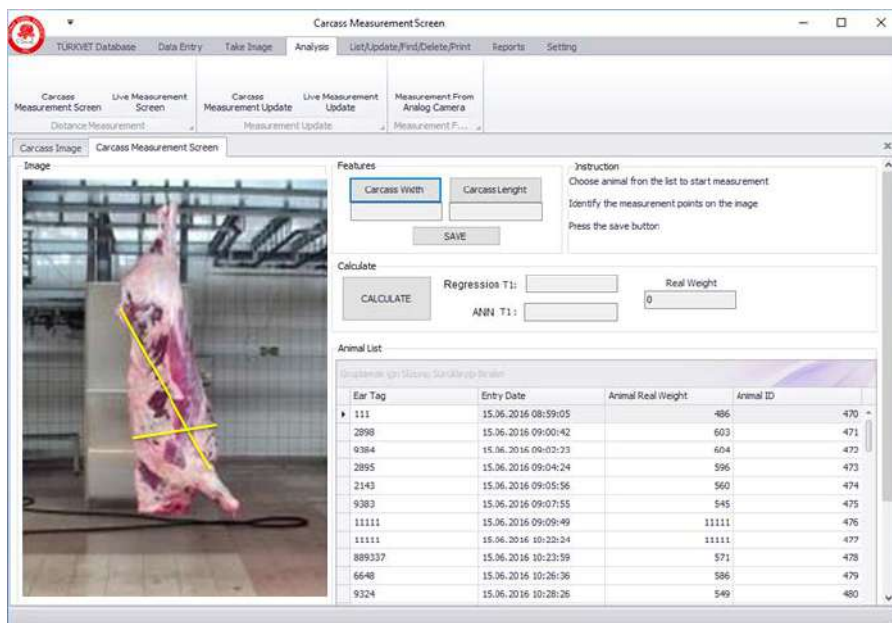


Figure 5. Carcass Measurement Screen

When the Carcass Measurement menu is selected, the Carcass Measurement Screen window in Figure 5 is displayed. The carcass photo of the animals in the Animal List is displayed. The carcass width is marked as a line with the mouse and the Carcass Width button is pressed to measure the carcass width. The same process is repeated to take the carcass length measurement. When the CALCULATION button is pressed, the weights calculated with the Regression and ANN mathematical models are seen in the Method1 and Method2 fields in the program according to the measured lengths. The predicted carcass weight value is appeared in the actual weight field. Pressing the SAVE button saves the data for this animal. The recorded animal information disappears from the list. This is a on-going process until the slaughter party is completed

Getting reports menu

From the Reports menu in Figure 6, on the daily basis the number of slaughtered animals, the amount of carcass meat (kg), dressing percentages, distribution by breeds and producer information (name and bank account information etc.) producer lists and Date of reports are obtained.

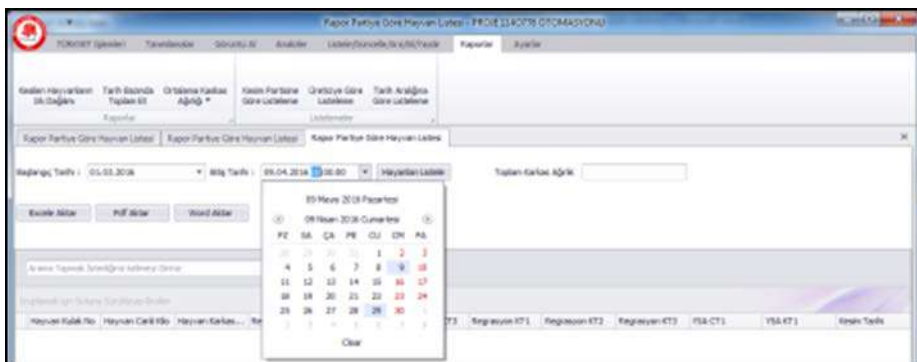


Figure 6. Report screen

Conclusion

In conclusion, the developed computerised system will be able to determine easily the carcass quality and meat yield characteristics and would be beneficial not only beef producers and abattoir management and it will speed up the slaughtering operations and increase the slaughtering capacity of the enterprises so that the information collected by the automation system can be tracked and recorded in a computer environment quickly and reachable easily wherever there is internet access.

In addition, recommendations for beef producers can be made by comparing carcass yields at each slaughtering times and how many times the same beef producer brought animals to the slaughterhouse within a year and discussing the reasons in carcass yield changes and feeding strategies such as ration adjustments according to the yield between the slaughtering periods.

Kompjuterizovani sistem za predviđanje klaničnih osobina goveda

Yalçın Bozkurt, Tuncay Aydoğan, Cevdet Gökhan Tuzun, Cihan Doğan

Rezime

Kompjuterski program razvijen je na osnovu modela linearne regresije (LR) i veštačkih neuronskih mreža (ANN) za predviđanje žive mase i nekih karakteristika trupa kao što su težina i prinos. Ovaj softver je povezan sa sistemom kamera, kada se koristi u farmama ili klanicama, mogu se instalirati na lične računare.

Modeli koje su LR i ANN razvili u prethodnim studijama korišćeni su za razvoj softverske aplikacije. Za testiranje, podaci su prikupljeni kao nezavisni podaci za prethodno zaklana goveda bez obzira na rasu i korišćeni su za validaciju modela i za određivanje performansi softverske aplikacije. Primećeno je da je sposobnost predviđanja modela procenjena na visokom nivou predviđanja sa granicom greške od $\pm 3\%$ za svaki trup.

Za validaciju modela, podaci bez obzira na rase prikupljeni su za goveda dovedena u privatnu klanicu. Sposobnost predviđanja modela je procenjena nakon visokih nivoa predviđanja sa opsegom greške od $\pm 3\%$ kg za svaki trup. Tada je razvijen kompjuterizovani sistem ne samo za klanice, već i proizvođače goveđeg mesa kako bi se pružile informacije o klaničnim karakteristikama goveda i poboljšale menadžerske veštine klanica.

Ključne reči: modeli predviđanja, veštačke neuronske mreže, linearna regresija, klanica

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THE EFFECT OF THE FLAX SEEDS NUTRITION OF CATTLE ON PRODUCTION AND SLAUGHTER PROPERTIES

Maja Petričević¹, Dušan Živković², Dušica Ostojić Andrić¹, Dragan Nikšić¹, Veselin Petričević¹, Marija Gogić¹, Violeta Mandić¹

¹Institute for Animal Husbandry, Autoput 16, 11080, Belgrade-Zemun, Republic of Serbia

²University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Belgrade-Zemun, Republic of Serbia

Corresponding author: Maja Petričević, majanovakovic@live.com

Invited paper

Abstract: The aim of the present study was to investigate the effect of adding flax seed to nutrition of cattle in the final stage of fattening. The sample included a total of 30 young bulls of Simmental breed of uniform initial weight, divided into 2 groups (control and experimental). The control group had no flax seed in the diet, while the trial groups of cattle consumed flax seed in the amount of 8.75% of the concentrated part of the ration, i.e. 700 g per day. The study included the examination of the fattening performance, slaughter properties, the share of the slaughter by-products and the composition of the bovine carcass. Post slaughter, individual measurements of warm carcass sides, with and without kidneys, were performed. After cooling, the left carcass side was cut into the basic parts according to the Rulebook. The results of this experiment showed that flax seed as a food supplement did not have a statistically significant ($p>0.05$) impact on the weight of young bulls at the end of the experiment. It also did not have a statistically significant ($p>0.05$) impact on differences in average total yield (ATY) of cattle and food conversion ratio. By cutting the carcass sides into the basic parts it was established that the flax seed diet did not influence significantly ($p>0.05$) the share of carcass parts or the share of the offals.

Key words: young bulls, Simmental breed, diet with flax seeds

Introduction

Farm animals represent an important resource for the economic development and nutritional security of a country (*Caput, 1994*). The world faces a constant increase of the population, which requires the production of enough food.

By selection and various methods of crossing, feeding, animal genetic resources have been promoted in the direction of higher and better production of animal products. Production conditions are increasingly controlled by man. In order to increase meat production in the Republic of Serbia, more productive breeds of cattle (Simmental, Holstein-Friesian) are favoured.

Meat is an important foodstuff of high quality in human diet. Beef has high nutritional value. It is a rich source of protein of high biological value, contains a low percentage of intramuscular fat, significant amounts of vitamin B complex and mineral substances (Wyness *et al.*, 2011). Of the total consumption of meat in the world, beef with a share of 24% is in third place, behind pork (36%) and poultry (33%). Beef meat has a higher price compared to other types of meat, and the low competitiveness of beef is conditioned by the long production cycle and by the higher consumption of food per kilogram of gain (Dokmanović *et al.*, 2014). According to FAO forecasts, by 2020, the production of beef will increase by 6.7% in developing countries and by 1.9% in developed countries (Anon, 2011).

Production factors (daily increase, food conversion ratio), physical and muscular development, as well as slaughter properties are cited as the main factors determining the efficiency and economy of production in beef cattle breeding. The quantity and quality of the final products depend directly on these factors. Based on data on body development, the total amount of meat can be determined with more or less accuracy (Ostojić-Andrić *et al.*, 2007). Carcass yield is a very important parameter of the quality of the cattle. It is influenced by breed (genetic basis), pre-slaughter weight, animal nutrition, type of meal (roughage or concentrated diet), conditions of transport and resting of animals in the slaughterhouse (Kralik *et al.*, 2007). On the other hand, the market is becoming an increasingly decisive factor determining the production of beef, especially in terms of quantity and quality. The market influences the direction of production of high-quality meat, certain taste and uniform structure and appearance for a product of defined quality, classified by the standards of individual countries. Nutrition, as well as the duration of fattening, are factors that significantly affect the quality of the carcass. The influence of nutrition on the nutritional aspect of beef quality has been gaining in importance in recent years.

Materials and Methods

The research was carried out on the experimental farm and in the experimental slaughterhouse of the Institute for Animal Husbandry in Zemun (Serbia). In the study, male cattle of Domestic Simmental breed were used. In the trial, 30 Simmental young bulls of uniform weights were selected, which consumed

the food of the same composition until reaching the age of 390 days. The feeding of cattle prior to trial was carried out according to existing diet composition norms for these cattle used on the farm of the Institute for Animal Husbandry (whole maize silage and concentrate mixture with 12% of the total protein). The fattening of young bulls was in the free system. In order to fulfil the trial objective, it was necessary to prevent the movement of the animals when consuming a concentrated portion of the meal, so that we can reliably claim that each animal consumed the predetermined amount of concentrate. At the age of 390 days, two groups of 15 cattle were formed: the control group (CON) in which the cattle did not consume heat-treated flax seed and the experimental group (LS) in which the part of the concentrate was replaced by heat-treated flax seeds, so that each animal consumed 700 g of flax seeds per day. The final pre-slaughter weights were about 580 kg. One day before slaughter, the bulls did not receive food, but they had free access to water. Slaughtering and primary processing were performed in the experimental slaughterhouse of the Institute for Animal Husbandry. Animals were measured immediately before slaughter and then slaughtered according to standard commercial procedures. After primary processing, the carcasses were placed in a cooling chamber at 4°C for the next 24 hours. The weight of the warm carcass, the weight of the intestines (heart, lungs, liver, kidneys, spleen and tongue), head, tail and kidney fat was measured one hour post-slaughter and treatment. After chilling, the carcasses were measured and split along the vertebral column in two halves, and the left side was used for all measurements. The left side of each carcass was divided into twelve anatomical regions: round, beefsteak, loin, shoulder, back, neck, chest, short ribs, ribs, flank, fore shank and leg, using a standard technique.

The obtained data were processed by analysis of variance in one-way ANOVA program SPSS Statistics 20, and all results are displayed as the mean value \pm standard deviation. The statistical significance of the difference between mean values was determined by t-test.

Results

The results for fattening and slaughter traits of young bulls are shown in Table 1. The use of flax seeds in the final stage of the fattening did not result in the difference between the groups in the average daily gain (ADG) and the food conversion ratio. Higher ADG as well as better food conversion were recorded in LS group, but the established differences were not statistically significant ($p > 0.05$). The values of the TT carcass yield and the TT weight with and without fat, as well as the CC carcass yield and the CC weight with and without fat, were higher in the CON group, but for the established differences there was no statistical significance ($p > 0.05$). For the other parameters listed in Table 1, the young bulls of the LS

group had higher values compared to the CON group, but also the differences were not statistically significant ($p > 0.05$).

Table 1. Average values of fattening and slaughter traits of cattle/young bulls

	CON	LS	p
ADG ¹ (kg)	1.59 ± 0.33	1.66 ± 0.19	ns
Feed conversion (kg)	7.23 ± 0.53	6.89 ± 0.29	ns
Weight PS ² (kg)	576.25 ± 25.36	585.00 ± 6.24	ns
Weight WC ³ with fat (kg)	337.52 ± 19.83	336.00 ± 12.83	ns
Yield WC ³ with fat (%)	58.56 ± 1.53	57.30 ± 2.53	ns
Weight WC ³ without fat (kg)	333.50 ± 19.33	331.67 ± 11.93	ns
Yield WC ³ without fat (%)	57.85 ± 1.32	56.70 ± 2.10	ns
Weight CC ⁴ without fat (kg)	326.82 ± 18.96	324.87 ± 11.45	ns
Yield CC ⁴ without fat (%)	56.70 ± 1.27	55.54 ± 2.00	ns
MLD ⁵ Cross section surface (cm ²)	100.95 ± 16.35	111.02 ± 12.28	ns
Kidney fat (%)	0.70 ± 0.24	0.74 ± 0.23	ns
CL Cooling loss ⁶ (%)	3.17 ± 0.44	3.31 ± 0.53	ns
Head (%)	2.59 ± 0.09	2.74 ± 0.20	ns
Tail (%)	0.17 ± 0.02	0.21 ± 0.02	ns

¹ ADG – average daily gain; ² PS – Pre- slaughter; ³ WC – Warm carcass; ⁴ CC– cooled carcass; ⁵ MLD – *Musculus longissimus dorsi*; ⁶ CL – loss of weight during cooling; ns – not significant

The influence of the flax seed based diet on the intestine content in the pre-slaughter weight is shown in Table 2.

Table 2. The effect of the addition of flax seed in the cattle diet on the share of intestines**

(%)	CON	LS	p
Kidneys	0.17 ± 0.02	0.19 ± 0.01	ns
Liver	1.01 ± 0.11	1.27 ± 0.17	ns
Lungs	0.55 ± 0.08	0.60 ± 0.07	ns
Heart	0.30 ± 0.02	0.34 ± 0.03	ns
Spleen	0.18 ± 0.03	0.22 ± 0.01	ns
Tongue	0.24 ± 0.03	0.29 ± 0.03	ns

**Relative to the pre-slaughter weight; ns – not significant

Table 3 shows the share of the basic carcass parts. The shares of the carcass parts were not statistically significantly ($p > 0.05$) different between the groups. The shares of beef steak and round were approximately the same in groups, with the bulls from LS group having higher values for the mentioned parameters. The share of round ranged from 28.05% in CON to 28.44% in LS. The higher values of the share of loin and back parts were recorded in bulls of LS group, whereas a higher share of the shoulder was found in the bulls of the CON group under the influence of flax seed.

Table 3. The effect of the addition of flax seed in the feeding of cattle on the share of main carcass parts *

(%)	CON	LS	p
Beef steak	2.41 ± 0.45	2.64 ± 0.37	ns
Round	28.05 ± 1.21	28.44 ± 0.83	ns
Loin	4.84 ± 1.15	5.26 ± 0.44	ns
Back	5.48 ± 0.69	6.00 ± 0.32	ns
Shoulder	12.60 ± 0.73	11.41 ± 0.78	ns
Leg	3.66 ± 0.52	3.81 ± 0.25	ns
Fore shank	2.78 ± 0.26	3.03 ± 0.12	ns
Neck	10.14 ± 1.01	9.83 ± 0.48	ns
Chest	5.18 ± 0.64	4.56 ± 0.56	ns
Short ribs	11.90 ± 0.43	13.31 ± 0.69	ns
Ribs	6.75 ± 1.43	5.61 ± 0.56	ns
Flank	6.16 ± 0.77	6.05 ± 0.74	ns

*Relative to the processed carcass; ns – not significant

Discussion

Nutrition with flax seed did not have an effect on production performance and slaughter properties, which is consistent with research in which flax seeds were added at different concentrations (*Barton et al., 2007* and *Raes et al., 2004* (extruded linseed); *Scollan et al., 2001* and *Mach et al., 2006* (whole linseed) and *Choi et al., 2000* (whole linseed treated with formaldehyde)). It is obvious that, based on our research and research of Barton et al. (2007), flax seed can be included in feeding at levels up to 1200 g/day without adverse effects on production and slaughter properties. According to *Petričević et al. (2011)* young bulls of Domestic Spotted breed of the Simmental type of average weight of 500 kg had a yield of 55.31%, and a cattle of average weight of 600 kg yield of 56.30%. In another study, *Petričević et al. (2015)* have found that the yield/dressing percentage of the Domestic Spotted breed of the Simmental type was on average 58.07%. *Maddock et al. (2006)* have shown that the inclusion of 8% of flax seeds in the diet improves the carcass properties but it can lead to an increase in the amount of fat that negatively affects some of the parameters of meat quality.

Higher carcass yields (57.8% and 57.7%) of bulls fed with mixtures containing 10% and 15% of flax seeds in relation to the group that did not consume flax seed (57.1%) are reported by *Kim et al. (2004)* and *Drouillard et al. (2002)*. These authors confirm with their research that flax seed is an acceptable source of lipids without adversely affecting the final fattening of the cattle. In general, the results of these studies are in accordance with the data presented in our study where

it has been established that flaxseed has no significant effect on the differences in the slaughter properties of the bulls' carcasses.

The area of the *M. longissimus dorsi* cross section (Table 1.) did not statistically significantly ($p > 0.05$) differ under the influence of the examined factor. Young bulls of group LS had a larger surface area of *M. longissimus dorsi*. The larger surface area of *M. longissimus dorsi* in the cattle that consumed the flax seed in the diet was determined by Rotta *et al.* (2009) and Quinn *et al.* (2008) in their research. The surface of the *M. longissimus dorsi* cross section was greater in cattle that consumed 8% of the ground flaxseed in the study by Maddock *et al.* (2006).

The weight loss after 24 hours of cooling did not differ between the examined groups (Table 1). This is confirmed by Hernández-Calva L.M. *et al.* (2011) and Petričević *et al.* (2019) who state that weight loss during cooling did not significantly change under the influence of flax seed diet.

The consumption of flax seed in the final stage of cattle fattening did not have an effect on the share of intestines in the pre-slaughter weight. The shares of all intestines were higher in the LS group compared to the CON group, but the differences were not statistically significant ($p > 0.05$). Petričević *et al.* (2019), in their study, provide similar values for the shares of intestines in bulls fed diet with and without the addition of flax seeds.

In the research by Petričević *et al.* (2015) the share of basic carcass parts of the bulls that did not consume flax seed in the diet were: round (28.36%), shoulders (12.20%), lower legs (3.59%) and forearms (2.73%). Petričević *et al.* (2019) state that the share of the main carcass parts did not differ between groups regardless of the addition of flax seed in the diet.

Conclusion

The results of this study have shown that the substitution of a part of the concentrated mixture with flax seed did not have a negative impact on the production performance and slaughter properties of young bulls at a quantity of 700 g/day. The shares of the most valuable parts of the carcass (beefsteak and round) were slightly higher in the LS group, which indicates that the use of flax seed in the diet has a positive effect on the carcass composition.

Uticaj ishrane junadi sa semenom lana na proizvodne i klanične karakteristike

Maja Petričević, Dušan Živković, Dušica Ostojić Andrić, Dragan Nikšić, Veselin Petričević, Marija Gogić, Violeta Mandić

Rezime

Cilj ovog istraživanja je da se ispita uticaj dodavanja semena lana u ishranu junadi, u završnoj fazi tova. Uzorkom je obuhvaćeno ukupno 30 junadi simentalske rase ujednačenih početnih telesnih masa, koja su podeljena u 2 grupe (kontrolna i ogledna). Kontrolna grupa junadi u ishrani nije imala seme lana, dok su junad ogledne grupe konzumirala seme lana u količini od 8,75% koncentrovanog dela obroka, tj. 700 g dnevno. Istraživanje je obuhvatilo ispitivanje rezultata tova, klanične karakteristike, udeo pratećih proizvoda klanja i sastava trupa junadi. Posle klanja izvršeno je pojedinačno merenje toplih polutki sa i bez bubrežnog loja. Nakon hlađenja leva polutka je rasecana u osnovne delove prema Pravilniku. Rezultati ovog oglada su pokazali da seme lana kao dodatak ishrani nije imao statistički značajan ($p > 0.05$) uticaj na masu junadi na kraju oglada. Takođe nije imao statistički značajan ($p > 0.05$) uticaj na razlike u prosečnom ukupnom prirastu (PUP) junadi i konverziji hrane. Rasecanjem polutki junadi na osnovne delove utvrđeno je da ishrana sa semenom lana nije značajano ($p > 0.05$) uticala na udeo delova trupa kao i na udeo iznutrica.

Ključne reči: junad, Simentalska rasa, ishrana sa lanom

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MORINGA OLEIFERA, AN ALTERNATIVE PROTEIN SOURCE TO SOYA IN PIG PRODUCTION?

Giuseppe Bee¹, Antonia Katharina Ruckli^{1,2}

¹Agroscope Posieux, Posieux, Switzerland

²University of Natural Resources and Life Sciences, Vienna, Austria

Corresponding author: Giuseppe Bee, giuseppe.bee@agroscope.admin.ch

Invited paper

Abstract: Soybean meal (SBM) is commonly used as the main protein source in pig diets. In countries where this protein source is not easily available, alternative protein sources, such as *Moringa oleifera* (MO) leaves, have been proposed for pig diets. The aim of the present study was to establish the impact of replacing SBM by MO in a finisher pig diet on growth performance, carcass and meat quality. For the study, 24 Swiss Large White pigs from six litters were selected at 66.7 kg BW and assigned within litter to two isonitrogenous and isocaloric finisher diets containing either SBM (7.2%) or MO (15.6%) as the major protein source. All pigs were reared in one pen equipped with four automatic feeders. They had *ad libitum* access to the diets. The pigs were weighed weekly, and individual feed intake and feeding behaviour was monitored daily. After 55 d of feeding, the pigs were slaughtered, and the organ weights, carcass and meat quality traits, including the fatty acid composition of the adipose tissue and intramuscular lipids of the longissimus thoracis (LT) muscle, were assessed. The body weight (BW) at slaughter was 8.4 kg lighter ($P < 0.01$) in MO than SBM pigs. The lower ($P < 0.01$) growth rate was a result of the lower ($P < 0.01$) daily feed intake, whereas feed efficiency was not ($P = 0.49$) affected. Hot carcass weight and carcass yield was lower ($P < 0.01$ for each) in the MO than in the SBM group. Due to heavier ($P < 0.06$) ham and shoulder and lower ($P < 0.01$) subcutaneous fat weight, lean meat percentage was almost 2% units greater ($P = 0.04$) in the MO than in the SBM. Expressed as a percentage, the hot carcass weight, relative liver, kidney and lung weights were greater ($P < 0.01$ for each) in the MO pigs. The LT of the MO pigs was less ($P < 0.01$) red and yellow, and ultimate pH was 0.1 units greater ($P = 0.02$). Water holding capacity, but not shear force, was impaired in the LT of the MO pigs, as drip and thaw losses were greater ($P \leq 0.02$). The adipose tissue of the MO pigs was less ($P < 0.01$) saturated, mainly because of lower ($P \leq 0.02$) stearic and palmitic acid levels and greater ($P \leq 0.03$) levels of linolenic, palmitoleic, vaccenic and eicosanoic acids. Similarly, the intramuscular fat was less ($P = 0.01$) saturated because of lower ($P \leq 0.04$) myristic, palmitic and stearic acid levels and greater ($P \leq 0.07$) levels of oleic, vaccenic and linolenic acids in MO pigs. In conclusion, despite the similar nutrient

content of the finisher diets, replacing SBM with MO not only impaired growth but also negatively affected important meat quality traits.

Key words: pig nutrition, protein source, *Moringa oleifera*, growth performance, meat quality

Introduction

Because of its elevated protein content and ideal amino acid profile, soybean meal (**SBM**) is the most important protein source used to feed grower finisher pigs. In line with increasing worldwide meat consumption and concomitantly increasing livestock production, the demand for SBM steadily increases. For instance, from 2007 to 2017, the amount of soybean produced increased by 61% from 220 to 353 Mtons (*Faostat*, 2009). Of these amounts, 80% were produced in the USA, Brazil and Argentina. To satisfy the growing demand, new land had to be obtained partly by deforestation of the rain forest or by increasing the yield using new breeds or (genetically modified) cultivars and by intensifying the way of production. This development has inevitable consequences, not only on production sustainability but also on the world market price of SBM. Finding alternative plant protein sources, especially in subtropical and tropical regions where a huge variety of plants exist, might help alleviate the demand for SBM. Among the many plants of these regions which could be used in pig diets, *Moringa oleifera* leaf meal (**MOLM**) is of special interest. The Moringaceae is a single genus family with 14 known species. Of these, *Moringa oleifera* (**MO**) is the most widely known and utilized species. It is native to sub-Himalayan regions of India but can now be found in many countries in Africa, Arabia, SE Asia, the Caribbean and Central America (*Ramachandran et al.*, 1980). *Moringa oleifera* is a fast-growing tree which can reach 12 m in height at maturity, yielding up to 120 t/ha/yr when planted densely for use as forage (*Makkar and Becker*, 1997). The leaves are highly nutritious, containing significant quantities of vitamins, macro and micro minerals and protein (*Makkar and Becker*, 1997; *Foidl et al.*, 2001). Based on the report of *Moyo et al.* (2011), MO is an interesting plant from an animal nutrition point of view as it contains an elevated crude protein (**CP**) content [303 g/kg dry matter (**DM**)] compared to other feedstuff (e.g. legume forages and oil cakes) and a similar essential amino acid-to-lysine ratio to that of SBM. As with many plants, MO contains secondary plant metabolites, which might act as anti-nutritional factors, and could therefore negatively affect the growth performance of growing animals. Unanimously, *Makkar and Becker* (1996) and *Moyo et al.* (2011) reported low levels of compounds such as polyphenols and saponins. Recent studies with finisher pigs suggest that MOLM can be successfully included in

grower finisher pig diets (Mukumbo *et al.*, 2014; Nduku *et al.*, 2014; Zhang *et al.*, 2019). However, in the study by Mukumbo *et al.* (2014) and in that of Zhang *et al.* (2019), SBM was not completely replaced by MOLM. Therefore, the objective of the present study was to compare the growth performance, carcass and meat quality of pigs fed with isonitrogenous and isocaloric finisher diets, where the main protein source was either SBM or MOLM.

Material and Methods

The Swiss cantonal Committee for Animal Care and Use approved all procedures involving animals.

Moringa oleifera

Moringa oleifera was cultivated in La Paz Centro on 0.2 ha (Nicaragua, 63 m.a.s.l, 12°19'54'' N, 86°40'7''W). The seed density for the leaves production was 1 million seeds per ha. Every 35 d, fresh and mature leaves were harvested and sun-dried until a moisture content of approximately 7% was obtained. The dried plant material was then vacuum-packed in plastic bags, shipped to Agroscope Posieux (Posieux, Switzerland) and milled prior to usage for the feeding trial. At arrival, the gross chemical composition of MOLM was determined.

Animals, housing conditions and diets

A total of 24 Swiss Large White pigs (12 females and 12 castrates) originating from six litters of the Agroscope sow herd were used. From weaning to the start of the experiment, the pigs were reared in the same pen under the same environmental conditions and offered restricted access (80% of *ad libitum*) to the same standard starter (weaning to 25 kg BW) and grower (25 to 60 kg BW) diet. At an average BW of 66 kg, the pigs were allotted within gender to the control (CO) and MO treatment and moved to two pens of equal size (17.35 m²). Each pen was equipped with an automatic feeder and an individual pig recognition system (Schauer Maschinenfabrik GmbH & Co. KG, Prambachkirchen, Austria) as described previously by Bee *et al.* (2008). The two pens were in the same room and connected by an alleyway and therefore accessible to all 24 pigs. The pigs had *ad libitum* access to the experimental diets for 55 d and were weighed weekly. In the grower period, one pig died due to an intestinal obstruction; therefore, only 11 pigs (5 barrows and 6 females) were available for the CO treatment.

One prerequisite for the diet formulation was that the CO and MO diets were isoenergetic and isonitrogenous and covered the nutrient requirements of finisher pigs according to the Swiss feeding recommendations for pigs (Agroscope, 2017).

Table 1. Ingredient and nutrient composition of the experimental control (CO) and Moringa (MO) supplemented diets and the nutrient composition of the Moringa oleifera leaf meal (MOLM) and soybean meal (SBM)

Item ¹	Finisher diet		MOLM ²	SBM ³
	CO	MO		
Ingredient, g/kg				
Barley	706.00	--		
Corn	48.06	4.40		
Wheat	32.26	684.50		
Potato protein	18.00	18.00		
Soybean extracted meal	71.80	--		
Moringa oleifera		155.58		
Wheat bran	96.62	87.78		
Dried sugar beet pulp	0.04	32.86		
L-Lysine-HCL	1.22	2.70		
DL-Methionine	0.02	0.10		
L-Threonine	0.36	1.02		
Dicalcium phosphate	0.82	1.96		
Limestone	12.80	2.04		
Sodium chloride	4.90	1.96		
Pellam	3.00	3.00		
Mineral-vitamin premix	4.00	4.00		
Natuphos 5000 G	0.10	0.10		
Analysed nutrient composition, g/kg of DM				
Crude fibre	50.83	45.27	89.76	88.00
Neutral detergent fibres (NDF)	191.01	162.41	130.46	139.81
Acid detergent fibres (ADF)	69.38	66.84	112.74	103.76
Hemicellulose	121.63	95.57	17.72	36.05
Crude fat	30.85	38.79	42.59	58.36
Σ of saturated fatty acids (SFA)	7.24	6.95	12.49	15.97
Σ of monounsaturated fatty acids (MUFA)	5.18	4.46	1.77	2.03
Σ of polyunsaturated fatty acids (PUFA)	17.85	16.76	25.94	21.26
Crude protein	161.93	156.83	240.21	495.17
Lysine	8.87	8.92	14.32	29.89
Methionin	2.58	2.74	4.41 (0.31)	6.95 (0.23)
Cystine	3.03	2.95	3.30 (0.23)	7.61 (0.25)
Threonine	6.51	6.52	10.41 (0.73)	19.09 (0.64)
Tryptophan	2.20	2.29	4.78 (0.33)	6.47 (0.22)
Isoleucine	6.47	6.00	11.11 (0.78)	21.74 (0.73)
Leucine	11.93	11.21	20.24 (1.41)	38.16 (1.28)
Phenylalanine	8.16	7.46	13.20 (0.92)	24.30 (0.81)
Valine	8.12	7.49	13.32 (0.93)	22.99 (0.77)
Tyrosine	2.20	2.29	9.21 (0.64)	17.38 (0.58)
Histidine	3.72	3.38	5.16 (0.36)	12.63 (0.42)
Alanine	7.07	6.74	14.12	20.94

Arginine	9.41	8.16	14.01	36.59
Asparagine	13.18	10.81	21.78	57.02
Glutamine	32.10	31.02	23.79	88.42
Glycine	7.02	6.80	12.36	20.71
Proline	13.38	12.30	15.66	23.30
Serine	7.30	6.23	9.25	24.13
Calcium	6.93	7.32	25.12	3.12
Phosphorus	5.10	4.90	3.63	6.69
Gross energy, MJ/kg			19.20	20.83
Calculated DE content, MJ/kg ⁴	14.4	14.4		

¹Pellan = Press adjuvant for the production of pelletized feed; Mineral-vitamin premix supplied the following nutrients per kg of diet: 4000 IU vitamine A, 400 IU vitamine D3, 65 IU vitamine E, 3.0 mg riboflavine, 3.0 mg vitamine B6, 0.020 mg vitamine B12, 1 mg vitamine K3, 15.1 mg pantothenic acid, 15 mg niacin, 0.5 mg folic acid, 200 g choline, 0.050 mg biotin, 20.1 mg Fe as FeSO₄, 0.15 mg I as Ca(IO)₃, 0.15 mg Se as Na₂Se, 4.0 mg Cu as CuSO₄, 55.1 mg Zn as ZnO₂ and 10 mg Mn as MnO₂; Expressed as a percentage of total fatty acids, the CO diet contained 23.62% SFA, 17.11% MUFA and 58.99% PUFA, and MO diet contained 23.54% SFA, 15.44% MUFA and 59.56% PUFA. Expressed as a percentage of total fatty acids, the main fatty acids of the CO diet were palmitic (20.36%), oleic (15.71%), linoleic (54.61%) and linolenic acids (4.38%), and those of the MO diet were palmitic (21.43%), oleic (13.87%), linoleic (46.53%) and linolenic acids (13.03%).

² The level of essential amino acid expressed in g/g lysine is in brackets.

³ Nutrient compositions were obtained from the Swiss Feed Database (<https://www.feedbase.ch>).

⁴ The digestible energy coefficients from each feed ingredient were obtained from the Swiss Feed Database (<https://www.feedbase.ch>), and taking into account the relative amount of each feed ingredient in the diet, the digestible energy content was calculated.

Furthermore, in the MO diet, the amount of SBM used in the CO diet had to be fully replaced by MO leaves meal. The ingredients used for feed formulation and the analysed gross chemical composition of the two diets are presented in Table 1. As it was not possible to obtain an adequate protein level using just MO leaves, potato protein was used in the same amount in both experimental diets.

Processing of feeding behavior data

The feeding system used in the present study recorded the number of daily visits at the feeder, feed intake (**FI**) per visit and time spent at the feeder. For data evaluation, only feeder visits which coincided with the intake of feed (but not sham visits) were considered. As proposed by *Carcò et al. (2018)*, the day, not the single visit, was considered the temporal basis to describe the feeding behaviour of the pigs in the experimental period. Thus, the total feed intake, total feeder visits and total feeding time per day per pig were calculated. From these data, the average total time feeding per day (**TTF** expressed in min), average frequency of feeder visits (**FFV**), average time per visit (**TV** = TTF/FFV), average daily feed intake (**DFI**), mean feed intake per visit (**FIV** = DFI/FFV) and mean rate of feed intake (**RFI** = FIV/TV expressed in min) were calculated.

Slaughter procedure

The pigs were slaughtered on three different dates (4, 11 and 8 pigs, respectively). It was guaranteed that the same number of pigs from each experimental group was included at each slaughter date. The day of slaughter, all pigs were transported (100 m) from the piggery to the research abattoir. Two pigs from each treatment were stunned together for 180 s using a CO₂ (87% CO₂) stunner (Samson C1 L 803, MPS group, Holbaek, Denmark). After being exsanguinated and scalded for 3 min at 70°C, the ear cartilages, bristles and eyes were removed. Subsequently, the carcass was eviscerated and split across the midline, and the hot carcass (whole and right side) and the heart, kidneys, lung and liver were weighed. Afterwards, the carcasses were chilled at 2°C for 24 h.

Carcass and meat quality

At 30 min and at 24 h post mortem (pm), the pH was measured in the longissimus thoraci muscle (**LT**) at the 10th rib level with a WTW pH meter (pH196-S; WTW, Weilheim, Germany) equipped with a WTW electrode (WTW Eb4, Weilheim, Germany) and a temperature probe. The pH electrode was calibrated at the beginning of each series of measurements and temperature adjusted according to the mean temperature of the LT. The day after the slaughter, the whole and right side of the cold carcass was weighed. The left side was then dissected into the valuable cuts as previously described (*Bee et al., 2004*). The fat thickness was measured at the 10th rib level of the back fat.

The LT samples from the right carcass side were obtained for determining colour and drip, thaw, and cooking loss as an indicator of water-holding capacity and shear force as an indicator of tenderness. From each pig, the LT was cut at the eighth rib level caudal into eight 1.5 cm thick slices and labelled A, B, C and D. The slices weighed on average 77 g. In addition, at the 10th rib level, a back fat sample was collected. Prior to assessing the L* (lightness), a* (redness) and b* (yellowness) values with the CM-2600 spectrophotometer (Minolta, Dietikon, Switzerland), the chop labelled A and the back fat were bloomed for 20 min. The A and C chops were then vacuum-packed, stored at 2°C for 4 d and then frozen at -20°C. Within 2 months, the chops were thawed for 24 h at 2°C to determine thaw loss. After 1 h at room temperature, the samples were cooked on a preheated (190 to 195°C) grill plate (Beer Grill AG, Zurich, Switzerland) to an internal temperature of 69°C to determine cooking losses. When the cooked samples reached room temperature, shear force was determined (five cores per chop) using a Stable Micro System TA.XT2 Texture Analyzer (Godalming, Surrey, UK) equipped with a 2.5-mm-thick Warner-Bratzler shear blade. For the data analysis, the mean of the 10 replicates per pig was used. Using the chop B and D, drip loss

during 24 h at 2°C was determined with the plastic bag method used by (Honikel, 1998). Subsequently, the fat was trimmed off these chops and then vacuum packed and stored at -20°C until a thiobarbituric acid reactive substances (TBARS) analysis was performed.

Chemical Analysis

The diets, MOLM, adipose tissue and LT analyses were conducted in duplicate except when the results differed by more than 5%, up to four replicates were obtained. The dry matter (**DM**) in the feed was determined by gravimetry, after drying at 105°C for 3 h. The dry matter content of the LT was determined by gravimetry after lyophilisation. The ash content was determined in the feed and MOLM samples after 3 h at 550°C. In the same samples, the crude protein (**CP** = total N \times 6.25) content was analysed with a LECO FP-2000 analyser (Leco, Mönchengladbach, Germany). The amino acid composition of the diets and MOLM was determined after 24 h of acid hydrolysis (48 h for leucine, isoleucine and valine). Methionine and cystine were hydrolysed after peroxidation with formic acid. The amino acid profile was determined by HPLC coupled with a fluorescence detector (*Alliance 2695; Waters, Milford, MA, USA*) as described in the manual (Waters AccQ Tag Chemistry Package 052874 TP, rev. 1). In the diets, crude fibre and ether extracts were determined according to the VDLUFA (2007) methods 6.1.4 and 5.1.1, respectively. The neutral detergent fibre (**NDF**) and acid detergent fibre (**ADF**) were analyzed according to standard protocols using the ANKOM200/220 fibre analyser (Ankom Technology Corp., Fairport, NY, USA). The NDF assay was done with heat-stable amylase and sodium sulfite and expressed without residual ash after incineration at 550°C for 1 h (ISO 16472:2006), and the ADF was determined according to *Van Soest (1963)* and expressed without residual ash. To determine the crude fat content in the feeds, the samples were hydrolysed in 10% HCl (v/v) for 1 h. The hydrolysate was dried and subsequently extracted with petrol ether by using the Büchi SpeedExtractor E 916 (Büchi Labortechnik AG, Flawil, Switzerland). The dry residual of fat was determined by gravimetry. The fat content in the freeze-dried samples was determined using the Avanti Soxtec System (2050 Extraction Unit; Foss Tecator, Hillerød, Denmark). In the MOLM, the gross energy content was measured with an adiabatic bomb calorimetry (ISO, 1988) using a LECO AC600 (Leco, St. Joseph, MI, USA). The fat content and fatty acid profile of the adipose tissue and LT was determined as outlined by *Ampuero et al. (2014)*. Briefly, lipids were transmethylated for 3 h at 70°C using 5% methanolic HCl as an acid reagent. The methyl esters formed were neutralized with a solution of potassium carbonate and purified on silica gel. Fatty acid methyl esters were analyzed by gas chromatography (6850 series; Agilent Technologies AG, Basel, Switzerland)

equipped with a flame ionization detector (detector temperature 250°C). Non-adeanoic acid methyl ester (19:0) was used as internal standard.

Statistical analysis

The data were analysed with the MIXED procedure of SAS (SAS Inst. Inc., Cary, NC) with the individual pig as the experimental unit. The model used for the data analyses of growth performance, carcass characteristics and meat quality traits included the feeding regime, sex and the feeding regime \times sex interaction as fixed effects, and the litter of origin as the random effect. For the carcass traits, the date of slaughter was included as an independent factor. Least square means were calculated, and the PDIFF option of SAS was used to determine the differences between dietary treatment groups. The differences were considered significant at $P < 0.05$.

Results and Discussion

Moringa oleifera, diet composition and growth performance

Compared to SBM, MOLM contained 51% less CP, 52% less lysine, 27% less fat and 8% less gross energy but similar amounts of crude fibres, NDF and ADF (Table 1). The essential amino acids concentration expressed as g amino acids per g lysine was, except for histidine, greater in MOLM than in SBM, which explains why this plant is regarded as a valuable substitute for soy (*Gopalakrishnan et al., 2016*). *Shih et al. (2011)* found similar CP and crude fat levels in MOLM from Taiwan to that used in the present study, whereas *Kambashi et al. (2014)* reported markedly greater CP, NDF, ADF and fat contents in MOLM from Africa. Not surprisingly, these data confirm that environmental and cultivation factors (e.g. the use of different sources of fertilizer) (*Dania et al., 2014*) play an important role for the nutrient content of MOLM. As in the present study, MOLM was analysed for its nutrient content and these values were used for feed formulation, it was expected that the gross chemical composition, the amount of essential amino acids and the level of the three main fatty acid groups would be similar in the CO and MO diets (Table 1).

Despite having *ad libitum* access to the isonitrogenous and isocaloric diets for 55 d, the MO pigs were 8.4 kg lighter ($P < 0.01$) at slaughter, ingested 21% less feed daily and grew 19% slower ($P < 0.01$) than the CO pigs. Replacing 7.2% SBM with 15.6% MO had no effect on feed efficiency, implying that growth was hampered primarily by the lower feed intake with no negative effect on nutrient conversion. In contrast, *Nduku et al. (2014)* observed increased feed intake when 7.5% MOLM was included in the finisher diet, but feed efficiency was impaired as the growth rate was not positively affected. Surprisingly, in the same study, when

the MO supplementation was lower (0, 2.5 and 5%), the growth performance traits were unaffected. In the study by *Zhang et al. (2019)*, the feed intake and growth rate linearly increased, but the feed efficiency decreased in a quadratic matter with increasing MO inclusion (from 0 to 9%) in the finisher diets. Thus, the differences in the response of feed intake and growth rate to dietary MO between the present and the aforementioned experiments could be in part attributed to the different inclusion levels of MO. In addition, some evidence suggests that the dietary MO supply altered the feeding behavior, as the frequency of feeder visits (-18% ; $P = 0.10$), time per visit (-12% ; $P < 0.01$) and rate of feed intake per min (-11% ; $P = 0.04$) were lower compared to the CO pigs. The results of recent studies have suggested that not only total consumption and but also consumption pattern are indicative for the palatability of feed (*Frias et al., 2016; Figueroa et al., 2019*). In the case of MO, one could consider the presence of anti-nutritional components as a reason to impair the palatability of the diet (*Martens et al., 2012; Achilonu et al., 2018*). According to *Makkar and Becker (1996)*, MOLM contains only negligible levels of tannins, trypsin inhibitors, lecithins and saponin, whereas the levels of phytate, known to reduce the availability of minerals, were elevated. Furthermore, MO leaves contain elevated levels of raffinose and stachyose, which are considered flatulence factors and known to reduce appetite (*Gupta et al., 1989; Makkar and Becker, 1996; Jezierny et al., 2010*). As in the present study the content of these possible anti-nutritional components were not analyzed, it remains speculative as to which of the factors were responsible for the reduced appetite. An additional nutritional factor known to limit feed intake and change feeding behavior is the level of fibrous feed components. When comparing the levels of crude fiber, NDF and ADF of MOLM, SBM and the two diets, the differences were non-existent or too small to explain the difference in total consumption and the consumption pattern.

As often reported (*Li and Patience, 2017; Ruiz-Ascacibar et al., 2019*), a marked difference was observed in the growth performance between female pigs and castrates (Table 2). The female pigs ingested 17% less ($P < 0.01$) feed, grew 11% slower ($P < 0.01$) but were 7% more ($P < 0.01$) efficient than the barrows. The lower feed intake is reflected in the altered feeding behaviour, as the female pigs went two times less often ($P = 0.10$) to the feeder on average and spent an average 11 min less ($P < 0.01$) time at the feeder than barrows, but the feed intake per visit and the time per visit were similar. As the treatment \times sex interactions were never significant for the growth performance and feeding behaviour traits, it can be interpreted that the deleterious effect of MO supplementation was similar in the females and castrates.

Table 2. The effect of dietary treatment and sex on growth performance and feeding behaviour

Item ¹	Treatment ²		Sex		SEM	P-value ³		
	CO	MO	Gilt	Barrow		T	S	T × S
Body weight, kg								
at the start	65.9	67.5	68.0	65.3	1.40	0.43	0.18	0.66
at slaughter	118.3	109.9	112.7	115.5	2.30	< 0.01	0.26	0.46
ADG, kg/d	0.95	0.77	0.81	0.91	0.030	< 0.01	< 0.01	0.17
Total FI, kg	185.54	147.73	151.26	182.02	5.873	< 0.01	< 0.01	0.36
ADFI, kg/d	3.37	2.69	2.75	3.31	0.107	< 0.01	< 0.01	0.36
G:F, kg/kg	0.28	0.29	0.30	0.28	0.005	0.38	0.01	0.28
G:DE, kg/MJ	0.022	0.022	0.023	0.022	0.0006	0.38	< 0.01	0.28
G:CP, kg/kg	1.98	2.08	2.10	1.95	0.038	0.06	< 0.01	0.26
Visits at the feeder, n								
FFV, n	11	9	9	11	0.9	0.18	0.10	0.82
Time spent at the feeder								
TTF, min	65	57	56	67	1.8	< 0.01	< 0.01	0.58
TV, min	7.1	7.2	7.2	7.1	0.59	0.91	0.85	0.55
Feed intake								
FIV, g	375	345	364	356	34.2	0.45	0.86	0.42
RFI, g/min	53	47	50	50	2.2	0.04	0.97	0.78

¹ ADG = average daily gain; FI = feed intake; ADFI = average daily feed intake; G:F = gain to feed ratio; G:DE = gain to DE intake ratio; G:CP = gain to crude protein intake ratio; TTF = average total time feeding per day (expressed in min); FFV = average frequency of feeder visits; TV = average time per visit (TV = TTF/FFV); FIV = average feed intake per visit (FIV = ADFI/FFV); RFI = average rate of feed intake (RFI = FID/TTF expressed in min).

² CO: control finisher diet; MO: finisher diet supplemented with 15.5% *Moringa oleifera* as the main protein source.

³ Probability values for the effects of dietary treatment (T), sex (S) and their interactions.

Carcass characteristics and morphometric measurements

As the BWs of the MO pigs at slaughter were 7% lower ($P < 0.01$) than that of the CO pigs, one could expect lower ($P < 0.01$) hot and cold carcass weights (Table 3). Furthermore, the carcass yield was 2.9% units lower ($P < 0.01$) and the percentage of cold loss was marginally greater (0.16%; $P < 0.01$) in the MO than in the CO pigs. The carcasses of the MO pigs were 1.9% units leaner ($P < 0.01$), mainly due to greater ham and shoulder portions, and contained 1.6% units lower ($P < 0.01$) amounts of subcutaneous fat.

Table 3. The effect of dietary treatment (T) and sex (S) on carcass characteristics and organ weights

Item ¹	Treatment ²		Sex		SEM	P-value ³		
	CO	MO	Gilt	Barrow		T	S	T × S
Hot carcass weight, kg	97.00	86.85	90.88	92.97	1.799	< 0.01	0.32	0.54
Cold carcass weight, kg	94.35	84.33	88.25	90.43	1.750	< 0.01	0.30	0.54
Carcass length, cm	103	102	103	102	0.8	0.20	0.25	0.97
Carcass yield, %	82.21	79.36	80.92	80.66	0.403	< 0.01	0.58	0.62
Cold loss, %	2.74	2.90	2.89	2.75	0.046	< 0.01	< 0.01	0.86
Lean meat, %	53.17	55.09	56.24	52.01	0.782	0.04	< 0.01	0.28
Loin, %	25.18	25.49	26.42	24.24	0.292	0.40	< 0.01	0.21
Ham, %	16.72	17.46	17.89	16.30	0.344	0.05	< 0.01	0.33
Shoulder, %	11.24	12.14	11.93	11.46	0.176	< 0.01	0.06	0.54
Belly, %	18.52	17.83	17.80	18.54	0.408	0.01	< 0.01	0.36
Subcutaneous fat, %	16.19	14.61	13.85	16.95	0.737	0.02	< 0.01	0.18
Back fat, %	9.99	8.85	8.41	10.42	0.529	0.02	< 0.01	0.45
Omental fat, %	1.96	1.78	1.55	2.19	0.160	0.31	< 0.01	0.72
Back fat thickness ⁴ , mm	23	19	17	24	1.3	0.02	< 0.01	0.09
Relative organ weight ⁵ , %								
Liver	1.87	2.21	2.04	2.05	0.057	< 0.01	0.81	0.48
Lung	0.67	0.78	0.77	0.68	0.028	< 0.01	0.02	0.48
Kidneys	0.36	0.40	0.40	0.41	0.014	< 0.01	0.74	0.28
Heart	0.45	0.48	0.48	0.45	0.008	0.01	0.02	0.79

¹ Carcass yield = hot carcass weight expressed as the percentage of the BW at slaughter; lean meat = sum of denuded shoulder, loin and ham weight as a percentage of cold carcass weight; subcutaneous fat = sum of external fat from the shoulder, back and ham expressed as a percentage of cold carcass weight; back fat = external fat from the loin expressed as a percentage of cold carcass weight; omental fat = omental fat weight expressed as a percentage of cold carcass weight.

² CO: control finisher diet; MO: finisher diet supplemented with 15.5% *Moringa oleifera* as the main protein source.

³ Probability values for the effects of dietary treatment (T), sex (S) and their interactions.

⁴ Measured at the 10th rib level.

⁵ Relative organ weight expressed as percentage of hot carcass weight.

The relative weights of the liver, lung, kidney and heart were greater in the MO than in the CO pigs. The heavier organs can only in part explain the lower carcass yield. The digestive tract plus the remaining digesta content must have been heavier in the MO pigs as well. From the current data, it is not possible to differentiate whether the digestive tract itself and/or the digesta were responsible

for the difference. However, as the feed was withdrawn from all the pigs 18 h prior to slaughter and the MO pigs ingested less feed daily, it might be reasonable to assume that the digestive tract was primarily heavier in the MO than in the CO pigs. Interestingly, *Serem et al. (2017)* reported numerically greater weights and concomitantly changes in the histoarchitecture of the spleen, liver and kidney of pigs fed a diet containing 12% MOLM for 7 weeks. The authors hypothesized that the anti-nutritional components of MOLM, especially at a greater dose, might have mild toxic effects when fed for a prolonged time.

Compared to the *ad libitum* feed allowance, restricted feeding affects fat tissue to a greater extent than lean tissue deposition when applied during the finishing period (*Lebret, 2008*). This explains why the MO pigs, who restricted themselves despite having *ad libitum* access to the feed, displayed leaner carcasses than the CO pigs. The same rationale explains why the carcasses of the gilts were leaner ($P < 0.01$) than those of the castrates. The greater carcass leanness was the result of greater ($P \leq 0.06$) percentages of loin (2.2% units), ham (1.6% units) and shoulder (0.5% units) and lower (-3.1% units; $P < 0.01$) portions of subcutaneous fat. The relative weights of the lung and heart were greater ($P \leq 0.02$) in the females than in the castrates. As for the growth traits, the carcass composition and morphometric measurements of the females and castrates were similarly affected by the diets, as the treatment \times sex interactions were never significant.

Meat and fat quality

At 24 h pm, the pH was 0.1 unit greater ($P = 0.02$) in the LT of the MO than that of the CO pigs (Table 4). The instrumental redness ($a^* = -0.8$), yellowness ($b^* = -0.6$) and chroma values (-1.0) were lower in the LT of the MO pigs. Compared to the LT chops of the CO pigs, the water-holding capacity determined as drip loss (-0.46% unit) and thaw loss ($+1.56\%$ unit), but not cooking loss, was lower ($P \geq 0.02$) in the MO pigs. The diet had no impact on the shear force values and the oxidative status of the meat. The observed differences were small despite being statistically significant. Nevertheless, except for greater a^* values in the LT of the pigs fed diets supplemented with up to 9% of MO in the study of *Zhang et al. (2019)*, no significant effects of MO on meat color, water holding capacity and shear force were reported in various other recent studies (*Mukumbo et al., 2014; Nduku et al., 2014; Zhang et al., 2019*). The most evident difference between the present study and those of *Mukumbo et al. (2014)*, *Nduku et al. (2014)* and *Zhang et al. (2019)* is the lower feed intake and growth rate due to MO. As reviewed by *Lebret (2008)*, feed restriction was in some cases reported to impair meat tenderness and juiciness because of lower intramuscular fat content.

Table 4. The effect of dietary treatment and sex on meat quality traits

Item	Treatment ¹		Sex		SEM	P-value ²		
	CO	MO	Gilt	Barrow		T	S	T × S
pH								
At 45 min pm	6.6	6.6	6.6	6.6	0.05	0.77	0.32	0.89
At 1 d pm	5.5	5.6	5.5	5.5	0.03	0.02	0.52	0.13
Temperature								
At 45 min pm, °C	38.4	38.4	38.3	38.4	0.39	0.96	0.57	0.79
Meat colour								
L* (lightness)	48.1	46.7	45.7	49.0	0.66	0.13	< 0.01	0.42
a* (redness)	5.8	5.0	5.1	5.7	0.18	< 0.01	< 0.01	0.30
b* (yellowness)	2.7	2.1	2.0	2.8	0.14	0.01	< 0.01	0.71
Chroma	6.4	5.4	5.5	6.4	0.20	0.01	< 0.01	0.35
Water holding capacity, %								
Drip loss (24 h)	0.89	1.35	1.26	0.99	0.177	0.02	0.13	0.32
Thaw loss ³	6.91	8.47	8.43	6.95	0.537	< 0.01	0.01	0.99
Cooking loss ⁴	21.91	23.14	23.08	21.97	0.707	0.61	0.09	0.85
Total ⁵	27.29	29.63	29.53	28.39	0.977	0.02	0.02	0.89
Shear force, kg	5.34	6.21	6.61	4.94	0.393	0.12	< 0.01	0.88
TBARS, MDA g/kg ⁶								
1 d post mortem	0.19	0.19	0.18	0.18	0.190	0.84	0.79	0.19
7 d post mortem	0.19	0.19	0.19	0.19	0.170	0.78	0.86	0.14

¹ CO: control finisher diet; MO: finisher diet supplemented with 15.5% Moringa oleifera as the main protein source.

² Probability values for the effects of dietary treatment (T), sex (S) and their interactions.

³ Weight loss during thawing for 1 d at 4°C.

⁴ Weight loss during cooking on a preheated (190 to 195°C) grill plate (Beer Grill AG, Zurich, Switzerland) to an internal temperature of 69°C. The slices were cooked for 1.5 min on one side, then turned and cooked for another 1.5 min, and finally on the initial side for an additional 1 min.

⁵ Total: sum of weight losses during thawing and cooking.

⁶ TBARS = thiobarbituric acid reactive substance; MDA = malondialdehyde.

The breed used in this study has an LT with an elevated intramuscular fat content, and the intramuscular fat content was not affected by the diets (Table 5). Thus, it does not help explain the differences in water holding capacity and meat color.

The LT of the gilts was darker ($L^* = -3.3$), less red ($a^* = -0.6$), less yellow ($b^* = -0.8$) and less vivid (chroma = -0.9 ; $P < 0.01$ for each). The drip and thaw losses, but not the cooking losses, were 1.48% units and 1.11% units greater ($P \leq 0.09$) in the females than in the castrates. With regard to tenderness, the LT from the gilts

had 1.67 kg greater ($P < 0.01$) shear force values than the LT from the barrows. The lower water holding capacity together with the lower instrumental tenderness measurements coincide with the markedly lower intramuscular fat content in the LT of the females compared to the castrates (Lebret, 2008). No effect of sex was found for the oxidative stability as evidenced by the similar TBARS values at d 1 and 7 pm. No dietary treatment \times sex interactions were observed for the meat quality traits, indicating that the impact of MO was similar for the gilts and barrows in terms of the growth and carcass traits.

Fatty acid composition of the adipose tissue and intramuscular fat

The adipose tissue of the MO pigs was 2.47% units less ($P < 0.01$) saturated, mainly because of the lower ($P \leq 0.02$) stearic and palmitic acid levels. On the contrary, the levels of PUFA and MUFA were 1.47% and 0.97% units greater ($P \leq 0.06$), respectively, due to the greater ($P \leq 0.03$) levels of linolenic, palmitoleic, vaccenic and eicosanoic acids in the MO than in the CO pigs. Similarly, the intramuscular fat was 2.26% units less ($P = 0.01$) saturated because of the lower ($P \leq 0.04$) myristic, palmitic and stearic acid levels. This lower level was compensated by greater ($P \leq 0.07$) levels of oleic, vaccenic and linolenic acids in the MO than in the CO pigs.

The adipose tissue of the gilts contained 6.4% less ($P < 0.01$) fat than the castrates and was 1.67% units less ($P = 0.04$) saturated because of the lower ($P \leq 0.03$) levels of myristic and palmitic acids. On the contrary, the PUFA level was 1.60% unit greater ($P < 0.01$) in the females than in the castrates, mainly due to the greater ($P \leq 0.04$) levels of linoleic and arachidonic acids. In accordance, the intramuscular fat content of the LT of the female pigs was 30% lower ($P < 0.01$), and the fat contained 2.10% less ($P = 0.02$) SFA and 2.68% units more ($P < 0.01$) PUFA than that of the castrates. The main differences in the individual fatty acids were observed for the levels of myristic, palmitic, linoleic and arachidonic acids.

Diet and the amount of deposited fat are major factors influencing the fatty acid composition of intramuscular and adipose tissue lipids, as suggested by Nürnberg *et al.* (1998). Increased PUFA uptake results in greater deposition of these fatty acids in the adipose tissues, and this effect is even more pronounced when *de novo* fat synthesis, and thus fat deposition, is decreased due to dietary constraints, such as restricted feed/energy intake (Bee *et al.*, 2002).

Table 5. The effect of dietary treatment and sex on fatty acid composition of the back fat and intramuscular fat

Item	Treatment ¹		Sex		SEM	P-value ²		
	CO	MO	Gilt	Barrow		T	S	T × S
Back fat								
Fat content	87.21	84.74	83.14	88.82	1.589	0.14	< 0.01	0.49
14:0	1.26	1.23	1.21	1.29	0.028	0.34	0.03	0.32
16:0	25.94	24.79	24.81	25.92	0.299	< 0.01	< 0.01	0.44
18:0	15.96	14.61	15.06	15.51	0.500	0.02	0.40	0.50
SFA	43.82	41.35	41.75	43.42	0.770	< 0.01	0.04	0.43
16:1n-9	1.82	2.06	1.91	1.96	0.105	0.03	0.60	0.89
18:1n-9	39.43	29.96	39.72	39.67	0.338	0.23	0.91	0.19
18:1cis-11	2.52	2.80	2.69	2.62	0.108	< 0.01	0.34	0.25
20:1n-9	1.14	0.87	0.99	1.02	0.078	< 0.01	0.52	0.85
MUFA	45.75	46.72	46.27	46.20	0.404	0.06	0.88	0.14
18:2n-6	8.70	8.88	9.48	8.11	0.389	0.65	< 0.01	0.59
20:4n-6	0.15	0.16	0.17	0.15	0.008	0.48	0.04	0.53
18:3n-3	0.59	1.63	1.17	1.06	0.060	< 0.01	0.13	0.26
PUFA	10.41	11.88	11.94	10.34	0.478	< 0.01	< 0.01	0.81
Fat content	2.87	2.51	2.26	3.21	0.233	0.28	< 0.01	0.91
14:0	1.20	1.08	1.09	1.20	0.040	0.04	0.06	0.40
16:0	24.64	23.13	22.97	24.80	0.347	< 0.01	< 0.01	0.42
18:0	12.30	11.62	11.87	12.04	0.366	0.03	0.55	0.36
SFA	38.30	36.04	36.12	38.22	0.677	0.01	0.02	0.35
16:1n-9	3.66	3.62	3.48	3.80	0.128	0.75	0.04	0.77
18:1n-9	42.93	43.95	43.43	43.45	0.543	0.07	0.97	0.18
18:1cis-11	4.52	4.71	4.61	4.62	0.195	0.08	0.97	0.28
20:1n-9	0.63	0.64	0.61	0.67	0.036	0.76	0.17	0.11
MUFA	52.17	53.41	52.60	52.97	0.742	0.07	0.57	0.15
18:2n-6	7.19	7.54	8.32	6.41	0.445	0.58	< 0.01	0.91
20:4n-6	1.55	1.66	1.88	1.33	0.153	0.62	0.02	0.60
18:3n-3	0.24	0.77	0.54	0.47	0.049	< 0.01	0.34	0.81
PUFA	9.35	10.42	11.22	8.54	0.653	0.25	< 0.01	0.81

¹ CO: control finisher diet; MO: finisher diet supplemented with 15.5% *Moringa oliefera* as the main protein source.

² Probability values for the effects of dietary treatment (T), sex (S) and their interactions

Despite observing greater PUFA in the tissue lipids after the dietary MO intake, the cause for this effect in the present study differed from that of the study of *Zhang et al. (2019)*. In the present study, the relative proportions of SFA, MUFA and PUFA were similar in the CO and MO diets, but the MO pigs ingested markedly less feed than the CO pigs. Based on the aforementioned considerations, one can therefore conclude that the PUFA enrichment in the adipose tissue and, numerically, in the intramuscular fat was due to the lower fat deposition in the MO pigs. Contrarily, *Zhang et al. (2019)* observed greater feed intake (on average 330 g/d) when pigs were fed 6% of MO. One can assume that this was the reason for the greater PUFA deposition. Interestingly, a conversion to long chain unsaturated fatty acids of the n-3 family was not observed in the present study, but occurred in the study of *Zhang et al. (2019)*. The reason for this discrepancy was not expected because greater n-3 fatty acid uptake usually increases the tissue level of eicosapentaenoic and docosapentaenoic acids. One possible explanation might be that the overall linolenic acid intake was insufficient to trigger linolenic acid elongation and desaturation.

Our findings that the level of unsaturation in the intramuscular fat and adipose tissue lipids was greater for the females than for the castrates confirmed the results of other studies (*Warnants et al., 1996; Bee et al., 2004*). In part, this effect can be attributed to the overall lower lipid content of tissues in females because of lower feed intake. Furthermore, when intramuscular lipid content is reduced, the proportion of unsaturated phospholipids is greater and results in an overall increase in PUFA content (*Bee et al., 2004*).

Because the dietary treatment \times sex interactions were not significant for the fatty acid profile of the adipose tissue and intramuscular fat, one can conclude that the observed changes in the fatty acid profile due to MO were similar for the gilts and barrows.

Conclusion

Moringa oleifera as a potential protein source for fattening pigs did not fulfil the requirements as a possible replacement for SBM. Besides decreasing the voluntary feed intake, carcass and meat quality were negatively affected. We hypothesize that the negative effects on the quality traits were a result of the lower ADFI of the MO diet. Compared to other aforementioned studies where MO supply was markedly lower, we can conclude that the concentration of 15.5% MO in the finisher diet was too elevated. However, to be a suitable replacement for SBM, such elevated levels need to be included. Future studies should focus on determining the exact reason for the lower palatability. If the effect is caused by anti-nutritional components, the preprocessing methods of MO should be tested.

The fact is that MO has a high potential in tropical areas as a protein source for monogastrics due to its rather elevated crude protein content and ideal amino acid profile.

***Moringa oleifera*, alternativa soji kao izvor proteina u proizvodnji svinja?**

Giuseppe Bee, Antonia Katharina Ruckli

Rezime

Sojina sačma (SBM) se obično koristi kao glavni izvor proteina u ishrani svinja. U zemljama u kojima ovaj izvor proteina nije lako dostupan, za ishranu svinja predloženi su alternativni izvori proteina, poput lišća biljke *Moringa oleifera* (MO). Cilj ove studije je da se utvrdi uticaj zamene SBM sa MO u finiše obroku na performanse rasta, kvalitet trupa i mesa. Za ovo istraživanje odabrane su 24 grla švajcarskog velikog jorkšira iz šest legla sa 66,7 kg telesne mase i podeljene su u okviru legla na dve izonitrogena i izokalorična finiše obroka koje sadrže ili SBM (7,2%) ili MO (15,6%) kao glavni izvor proteina. Sve svinje su bile uzgajane u jednom boksu opremljenom sa četiri automatske hranilice. Imali su *ad libitum* pristup obrocima. Svinje su merene nedeljno, a pojedinačni unos hrane i ponašanje tokom hranjenja nadgledani su svakodnevno. Nakon 55 dana, svinje su zaklane i procenjene su težina organa, trupa i kvalitet mesa, uključujući sastav masnih kiselina masnog tkiva i intramuskularne masti mišića *longissimus thoracis* (LT). Telesna masa (BW) na klanju bila je za 8,4 kg manja ($P < 0,01$) u MO od SBM svinja. Niža ($P < 0,01$) stopa rasta bila je rezultat nižeg ($P < 0,01$) dnevnog unosa hrane, dok na efikasnost hrane nije bilo uticaja ($P = 0,49$). Težina toplog trupa i prinos trupa bili su niži ($P < 0,01$ za svaki) kod MO grla u odnosu na SBM grupu. Zbog veće težine ($P < 0,06$) buta i plećke i manje ($P < 0,01$) težine potkožnog masnog tkiva, procenat nemasnog mesa bio je skoro za 2% jedinice veći ($P = 0,04$) u MO nego u SBM. Izraženo u procentima, težina toplog trupa, relativna težina jetre, bubrega i pluća bila je veća ($P < 0,01$ za svaku) kod svinja MO. LT kod MO svinja je bio manje ($P < 0,01$) crveno-žut, a krajnji pH je bio za 0,1 jedinica veći ($P = 0,02$). Sposobnost/kapacitet zadržavanja vode, ali ne sila presecanja, smanjen je kod LT svinja, pošto su kalo i kalo odmrzavanja bili veći ($P \leq 0,02$). Masno tkivo svinja MO bilo je manje ($P < 0,01$) zasićeno, uglavnom zbog nižih nivoa ($P \leq 0,02$) stearinske i palmitinske kiseline, i većih ($P \leq 0,03$) nivoa linolenske, palmitoleinske, vakcenske i eikozanske kiseline. Slično tome, intramuskularno

masno tkivo je bilo manje ($P = 0,01$) zasićeno zbog nižih nivoa ($P \leq 0,04$) miristične, palmitinske i stearinske kiseline i većih ($P \leq 0,07$) nivoa oleinske, vakcenske i linolenske kiseline u svinjama MO. Zaključno, uprkos sličnom sadržaju hranljivih sastojaka u finišer obroku, zamena SBM sa MO ne samo da je oslabila rast, već je negativno uticala i na važne osobine kvaliteta mesa.

Ključne reči: ishrana svinja, izvor proteina, *Moringa oleifera*, performanse rasta, kvalitet mesa

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LAYING HENS MANAGEMENT AND NUTRITION FOR MAXIMAL EGG PRODUCTION AT 100 WEEKS OF AGE

Miloš Lukić, Zdenka Škrbić, Veselin Petričević, Vesna Krnjaja, Zorica Bijelić, Nikola Delić

Institute for Animal Husbandry, Autoput 16, 11080, Belgrade-Zemun, Serbia
Corresponding author: Miloš Lukić, miloslukic.izs@gmail.com
Invited paper

Abstract: In the third decade of the 21st century, starting from 2020, leading breeding companies promise that their modern hybrids of laying hens will be genetically able to produce 500 eggs per hen till 100 weeks of age. This paper discuss some important questions about what we need to know and practice in the management, and especially in the feeding of these highly sophisticated, extremely productive and very demanding birds to achieve that goal in near future. The management and nutrition strategies, needs and recommendations, dynamics of feeding during the day and during the production cycle are discussed. The maximum production results in line with genetic predisposition can be achieved by precision nutrition, as well as by maintaining and preserving the health of laying hens and the quality of egg shell with proper nutrients and specially formulated diets, but first with closer cooperation between breeders, nutritionists and egg producers.

Key words: laying hens, maximal laying capacity in one cycle, feeding technology, feeding techniques

Introduction

Soon it will be possible to keep modern hybrid layers for 100 weeks, instead of 70, 80 or 90, with production of nearly 500 eggs per hen, as a result of the constant efforts primarily of the breeding companies, but also the table egg producers and the feed industry. The current management guide of the ISA Brown hybrid manufacturer state that today, in the optimal housing and nutrition conditions, we can expect 420 eggs per housed hen to the 90th week of age (ISA, 2018). The company's goal by 2020 is that their genetics in the extended production cycle of 100 weeks will be able to lay 500 quality eggs per housed hen. It is similar to other leading breeding companies.

Keeping of laying hens in continuous extended production up to 70-80 weeks without molting is still not a widespread practice. However, it is in the focus as the future direction of development of the breeding companies and some egg manufacturers due to potential economic and ecological benefits (*Bain et al., 2016*). On the other hand, induced molting and the second laying cycle as the standard way of extending the production life of layers is still a very common practice, for example, in the USA by estimates in about 20% to as much as 80% of table egg layers, depending on the specific year and conditions (*Flock and Anderson, 2016*). The decision whether the producer will extend the period of the flock exploitation and how depends on many factors, primarily those of production and economic nature.

Genetic progress and a longer production cycle are necessarily accompanied by changes in nutrition. Layers convert nearly one-third of daily consumed nutrients into eggs almost every day, which is a major daily metabolic effort, especially for the liver, bones and reproductive organs. In order to be able to adopt, metabolize, and then excrete large quantities of nutrients in a laid egg, hybrid layers require appropriate nutrition technology and precise daily satisfaction of feed requirements throughout the entire production cycle, where optimal conditions and healthy animals are implied.

In order to achieve high production in an extended cycle of exploitation of laying hens, it is necessary to apply as precision and as proper nutrition as possible. Precision nutrition is way and tools how best to achieve production goals (*Lukić et al., 2016*), and the primary goal of table egg producers is to maximize the use of genetic potential of the layers at the lowest price, i.e. in terms of nutrition, less nutrients in the manure and the more in the eggs. An additional important goal in the extended production cycle is to preserve the animal health and the quality of eggs as long as possible with proper nutrition. In order to achieve this in practice, a consistent application of the existing scientific information on animal nutrition is required, with a mandatory high level of control and rapid reaction to changes, as well as close cooperation between animal breeders, nutritionists/feed producers and egg manufacturers.

This invited paper will discuss important aspects of the management, especially the nutrition of the laying hens from the beginning of the laying period to the end of the extended exploitation, assuming that the rearing and transfer of pullets (the basis on which the future good laying capacity is built) was carried out on a proper way, and consequently aware of the fact that the extended production cycle actually begins on the first day of birds life. The paper aims to contribute to solving the challenges that are expected through the broader use of longer continuous exploitation of laying hen hybrids genetically capable for this.

Management and nutrition strategy

Decision whether the producer will extend the period of the flock exploitation and how depends on many factors, first of all economy ones (feed/egg price, possibility to sell spent flock and/or to buy a new one). Also, two basic production criterions that determines when to replace the (healthy) flock are reduced laying capacity and poorer quality of the eggs (especially the quality of the egg shell), which normally drops with the age of the layers (*Bain et al., 2016*). Therefore, for every successful extension of the laying cycle over 70 weeks of age, it is essential to preserve the optimum "working conditions" of all organs and tissues involved in egg production throughout the lifetime of the layer, primarily through proper management and nutrition strategy.

The basic nutrition strategic issue, whether to produce feed or partly or fully procure it on the market, is determined by the choice and capabilities of the egg producer. When choosing a feed manufacturer, in addition to the price and quality of feed as standard criteria, the scope and quality of customer support services are becoming increasingly important. The complexity and number of issues that need to be solved in the nutrition of modern layers to achieve maximum productivity justify the necessity and importance of teamwork and the need for a nutritionist in the team. Today, the basic questions of animal nutrition (what feed to use; how to feed modern animals in order to meet the needs of each individual in the flock; balance the ecosystem, producers' wishes and consumer demands) seem to be more and more difficult to find the right answers to. On the other hand, the multi-decade-long intensive development of the global feed industry, which has reached a total production of about 1 billion tons in recent years, has been successfully following the development of poultry industry by offering innovative solutions in the form of products and services (*Crots, 2016; Bradford, 2016*).

The availability of knowledge, experience, tools or services required, but first of all, willingness of the producers to change, could be a limiting factor for the wider global application of this (as well as any other) new practice. However, which management and nutrition strategy will be adopted and applied in future depends primarily on the goals and capabilities of each individual egg producer.

Precise and correct nutrition during (extended) laying cycle

First of all, the most recent data on the genetic potential and specific nutritional (and other) requirements of hybrids are needed. The best breeding companies are trying to summarize the decade-long practical and research experience of work with their own genetics, as well as (now) a century long

experience of the science of animal nutrition, in these manuals for breeding and nutrition. However, since the recommendations are nevertheless general estimates and averages, for the nutrition program of a particular flock it is necessary to adjust these values and specifications in order to calculate the appropriate reduction or increase of nutritional needs based on the actual production conditions and status of the flock, especially when the goal is to achieve as long a production cycle as possible. Also, new scientific knowledge should be constantly monitored.

Nutrition programs for modern hybrid layers of table eggs during the extended production cycle determine phase nutrition, as a practical way to keep constant changes in the lifecycle and production cycle accompanied by adequate dietary changes. In the case of laying hens, we can conditionally distinguish three life and technologically important periods: rearing (growth), rearing and production (simultaneously) and production. After rearing and feeding in three phases (starter, grower and developmental mixture), ending with the pre-laying mixture, hybrid producers recommend that layers during the exploitation are fed with three, four or even five different mixtures. Therefore, in order to formulate nutrition and diets for modern layer hybrids, eight different specifications of complete mixtures (from 5-10 mixtures, depending on the recommendations of the hybrid manufacturer) usually must be prepared in practice starting from the first day of life until the end of the production cycle.

The daily intake of nutrients (g of nutrients per hen per day) must be adequate to the daily needs of the layer of a given age, in specific farming conditions and with the achieved level of production. Given that adult laying hen normally have a ability to adjust their feed intake to their needs, primarily the energy, consumption of feed of each layer can vary, and is conditioned mostly by the body weight, production and concentration of energy in the mixture, as well as ambient conditions (temperature, humidity, gases) and the status of the individual layer (health, feathering, activity), especially if they are not optimal. The quality and form of the feed for layers can also significantly affect consumption if they are not adequate, especially the quality of the raw materials used, the cellulose content and the size of the particles in the mixture. Particular attention should be paid to the beginning of laying period, from 18-25 weeks of age when the needs increase rapidly (*Rutten, 2016*), and the ability of birds to consume slowly reaches its maximum (during this period young layers increase the consumption by nearly 40%). Daily control of feed intake and laying capacity, weekly control of body weight and weight of eggs and timely and adequate correction of the mixture in this crucial period establishes the balance of consumed and needed energy and nutrients in the organism of young layers, thus providing the necessary foundation for the long lasting laying of quality eggs.

Recommendations for the energy value of mixtures for layers are the starting point in the formulation of the diet because the adult poultry take feed until they satisfy their energy needs as primary, and usually all other nutrients are balanced against the level of energy input. In estimating the optimal diet energy, in addition to the recommendations, more information is needed to require, determine or adapt, in particular: to require all factors influencing the energy needs; to determine the precise production and environment conditions; to adapt (if necessary) energy recommendations to specific conditions and flocks. After completion of the growth and fulfilment of the body weight of adult layers, and after achieving the desired weight of eggs, which can be also influenced by the energy density of the diet, the energy of the mixture can be reduced as needed. The specifications of the need for macronutrients (proteins - amino acids, calcium, phosphorus, sodium, chlorides, linoleic acid and choline) for each hybrid are generally recommended in detail for each phase of nutrition and are shown in relation to feed consumption, so it is easy to adapt their level in the diet to the expected and achieved average feed consumption (energy) for a particular flock.

Monitoring of the flock in regard to meeting of the amino acid requirements and the fine-tuning of diets for adult layers is done based on the daily intake of amino acids and determined average daily weight of eggs. However, to which extent the individual needs of layers have been met depends on the achieved physical and productive uniformity of the flock, which decreases with the age of the flock. Therefore, in practice, the diets can be formulated with safe + 5% relative to the recommended amino acid concentrations (*Rutten, 2016*), if provided that the final production target (higher profit) with this change will be achieved. At the same time, in adult layers at the end of the laying period, the precision in the dosage of the diet proteins to some extent can control the optimum egg size, i.e. the quality of the egg shell, given that, according to *Bouvarel et al. (2011)*, 1 g of extra protein daily consumed by hen can increase the weight of the eggs by an average of 1.4 g.

For layers in the extended production cycle are very important the content and composition of fat and fibre in the diet for preservation of the health of the digestive tract (microbiota) and the liver, as well as precise consumption of calcium to preserve the quality of the egg shell and bones, and this must be paid special attention to.

Linoleic acid, essential fatty acids of well-known significance and influence, is recommended in the amount of at least 1% in the layer diet, which in practice is easily reached nutrition aspect. The problem with layers which has been in focus of research studies with limited success is the reduction of metabolic disorders and liver disease (hepatic steatosis – HS, fatty liver haemorrhagic syndrome - FLHS), to which modern layer hens are particularly susceptible. Hence,

many studies point to the importance of optimal, precise nutrition and energy throughout the entire production cycle of layers, as well as the replacement of a certain amount of carbohydrate with oils as a preferred source of energy for adult layers, in order to reduce the metabolic stress of the liver. In the recommendations of the hybrid manufacturers, the content of raw fats in the mixtures for layers, depending on their age, ranges between 2-5%, with the emphasis on the use of quality raw materials and antioxidant additives.

The importance of insoluble diet fibres (cellulose, lignin) in the extended laying period is increasingly emphasized, especially in two life periods of hybrids. In the period of rearing, it is recommended to include in the developmental mixture at least 5-6% due to better development of the digestive tract and increase of the ability to consume feed in young birds (*Lohmann Tiezucht, 2016*). The general recommendation is that 2.5-5% of insoluble fibres should be provided in the mixtures for hens during the laying period, based on numerous studies that showed their positive effect primarily on the digestive physiology and health of the microbiota of intestines (*Hendrix Genetics, 2018*). For older layers, moderate energy dilution of the diet is recommended by increasing the percentage of crude (insoluble) fibres to 6-7% in the mixture, which indirectly can positively affect the liver health, absence of incidences of cannibalism and a better consistency of faeces.

Prevention of osteoporosis in older layers and especially preservation of shell quality by nutrition should not be ignored when the laying cycle is extended. For the expected 500 eggs in the extended continuous laying capacity, the hen must be able to extract and incorporate in the shell over 1 kg of calcium. Hence, the significance and all aspects of nutrition and metabolism of calcium in laying hens have been intensively researched for decades, described and optimized in practice by a series of applied solutions and techniques (e.g. *Bouvarel et al., 2011; Lukić et al., 2011; Thiele et al., 2015*), but still excites researchers' interest. In general, adequate nutrition of layers with calcium implies the right solution for three essential diet aspects: the adequate source of calcium in the appropriate form and ratio (ratio of powder : 1.5-4mm granules); an adequate level depending on the age and feed consumption of the layers; the proper feeding technique to make calcium available at the right time and the right place in the body of the layer during the laying period. Attention should also be directed towards the adequate source and concentration of other minerals and vitamins essential for the formation of egg shell (*Nys et al., 2018*).

The recommended concentrations of micronutrients - vitamins and microelements, which are usually incorporated in diet by premix, are generally similar for adult layers in most hybrid manufacturers. However, if eggs with an increased content of certain microminerals or vitamins are production goal,

additional and precise fortification of the diet with certain micronutrients is necessary (Bouvarel *et al.*, 2011; Wang *et al.*, 2017; Nys *et al.*, 2018). Also, certain modification of used amount or form of some micronutrients and vitamins, as well as some additives inclusion can also have beneficial impact on liver health and the prevention of metabolic diseases. The inclusion of inositol in feed, vitamin D in the form 25 (OH) D₃ or certain herbal extracts, has shown potential to reduce oxidative stress of the liver and the occurrence of HS and FLHS in layers, and in practice, a certain increase in choline concentration, vitamin K₃, B₁₂, folic acid and vitamin E is implemented by incorporating in the feeds of one of the products for the preservation of liver health based on the combination of these vitamins (Pottgüter, 2016; Wang *et al.*, 2017).

In general, scientific research has so far identified nearly 40 nutrients that need to be provided to laying hen through diet, in optimal amounts, quality and interrelation. Usually, 4-5 (if necessary more) of the pronutrients and additives are added to the layer mixtures, in order to improve performance and to solve potential or current problems. That is why the proper diet of modern layer hybrids is considered one of the most demanding tasks in production, especially since there is no more standardized way of poultry farming, but a whole range of different ways and conditions for the production of table eggs, including the fact that the continuous laying cycle of the flocks is getting longer. Moreover, the question of full relevance of previous research, knowledge and recommendations, as well as their applicability can be raised without additional research and adaptation of the technology of feeding to new genetics, new conditions and new challenges – extended production cycle.

Dynamics and method of daily feeding

Layers can be feed *ad libitum* with well balanced diet because they are able to adjust the intake of feed to their needs and the density of nutrients in the mixture, and usually there is no overfeed. However, this mechanism is not completely precise. In particular, there is a danger of lower feed intake or imbalance of certain nutrients, such as amino acids or calcium, especially if there is an imbalance of energy and other nutrients in the diet, or in the diet with heterogeneous flour mixtures from which layers prefer to select larger particles.

Given the extremely high intake and excretion of nutrients in high-productive layer hybrids during each production day, it is important to properly satisfy the needs for particular nutrients even during the different parts of the daily cycle. The formation of eggs in the oviduct after ovulation, i.e. single ovulation cycle (from ovulation to oviposition) on average in modern layers lasts about 24 hours (Nys and Guyot, 2011). The ovulation is preceded by long multi-phase

development of the egg cell and the formation of the yolk in the ovary, with about 98% of the contents of egg yolks being deposited linearly during the final 7-11 days before ovulation, primarily lipids and proteins synthesized in the liver.

Most layers lay eggs (oviposition) in the morning, most often during the first hour after the start of the light part of the day cycle. During the formation of eggs, the first hours after ovulation albumen and egg membranes are formed, most often in the morning hours, followed by over 19 hours of formation and mineralization of the egg shell, mostly in the afternoon and at night (the most intense 10-22 hours after ovulation). It is therefore considered that in the case of layers, in the morning (oviposition, ovulation and formation of albumin), energy and amino acid requirements may be increased. Also, it has been proven that the need and appetite for calcium is rapidly increasing in the afternoon and at night (formation of the egg shell).

The right way of daily feeding should ensure that the necessary nutrients are available at the right time during the day for absorption in the intestines of highly productive layers. Therefore, hybrid manufacturers recommend feeding at least twice a day. A smaller portion (40%) of the total daily diet is given during morning feeding, and 60% in the afternoon, six to seven hours prior to turning off the light, which is a recommendation based on observation of the usual behaviour and appetite of layers during the day. It is also necessary that feeding be organized so that layers consume all the feed, so that the feeders are empty in the short time between these two feedings, to ensure that the smallest feed particles are consumed.

New method of feeding are studied, so-called “sequential” layer nutrition, in order to more precisely satisfy the needs during the daily production cycle. The feeding method of young layers was studied, where they were fed in the morning with an energy-fortified mixture, and in the afternoon with a mixture rich in proteins, calcium and micronutrients, which resulted in better utilization of feed and quality of egg shell with unchanged productivity, but accompanied by weight loss due to less consumption (*Batonon et al., 2014*). *Crots (2016)* reports the positive effects of specially designed sequential feeding in practical conditions for a successful extension of the production cycle, while *Molnar et al. (2018)* summarize all previous research on this subject in excellent review paper. In practice, further research is needed to apply this nutrition technique in practice, in particular its effects in the extended exploitation of coming hybrid layers.

The dynamics of changing of the mixture formulation during the production cycle – is improvement possible?

Some hybrid manufacturers suggest that it is necessary for the nutrition program to be as simple as possible with a limited number of feed changes in order to avoid mistakes in the layer nutrition chain (*Hendrix Genetics, 2018*), while others recommend that every 10 weeks during the production period the formulation of diet is adjusted to the current level of production and given recommendations for layer requirements (*Lohmann Tiezucht, 2016*). However, both warn that major changes in the raw material composition, quality and form of diets should be avoided, since layers are sensitive to rough changes in the basic characteristics of the diet.

Having constant, high-quality production monitoring is a prerequisite for success, as well as so-called “fine tuning”. It is not enough to just collect data, but the data collected must be real: relevant, representative, reliable and continuously monitored. Also, data without sound analysis and adequate and timely action mean nothing.

In order to improve the nutrition of layers, we can go further than that, which requires close cooperation between egg producers and feed producers. By constantly monitoring the complete production process and the quality of the product (egg producer), creating a quality database and expert analysis (a nutritionist or a feed manufacturer), primarily of the relationship between feed and production (the quality of feed ↔ the consumption ↔ intake of nutrients ↔ the response of layers ↔ the quality of eggs), errors can be avoided, and the nutrition program constantly optimized, if necessary with each subsequent delivery of feed, which is a new level of animal feeding - a proactive precision nutrition. It requires knowledge and experience of functioning of both systems, the (industrial) processes outside the hen, as well as in the biological systems (organism and organs) of the bird. New tools, application of sensors, information technology and modelling in the control and analysis of production, products and animal feed greatly facilitate work and widen the opportunities.

We need to find a measure, in larger interventions and frequent feed changes (to which the layers need to adapt), as well as in the method of applying precision nutrition, which should not be too complicated in order to remain practical and acceptable to a large number of feed manufacturers and table egg producers. On the other hand, the more accurate and precise nutrition of the layer, the greater are the chances that the genetic potential and resources are optimally used, i.e. to have less nutrients in the manure and more in the eggs.

The already mentioned sequential nutrition is being investigated and increasingly discussed, as well as the free choice method, as an easy way for each layer in a flock to be independently and in a natural way balance its daily ration (*Molnar et al., 2018*). These nutrition techniques could be widely accepted in the future, but with further necessary intensive research and development.

Conclusion

Adequate and high-quality nutrition of modern layers keeping on 100 week marathon of production of table eggs has a decisive impact on that how successful and for how long the high laying capacity, good quality of the eggs, and the animal health will be maintained. For maximum production results, so-called "one-size-fits-all" feed solutions are not sufficient. They are possible by the use of precision nutrition and specially formulated rations for specific conditions and flocks. The precondition for this is the multifactorial approach and close cooperation between breeders, nutritionists and egg producers.

Gajenje i ishrana kokoši nosilja 100 nedelja za maksimalnu nosivost

Miloš Lukić, Zdenka Škrbić, Veselin Petričević, Vesna Krnjaja, Zorica Bijelić, Nikola Delić

Rezime

Savremene hibridne nosilje uskoro će biti moguće držati bez mitarenja do 100 nedelja, umesto 70 ili 80, sa produkcijom blizu 500 jaja po nosilji. U radu se raspravlja o najnovijim saznanjima o gajenju i ishrani ovih visoko sofisticiranih, izuzetno produktivnih i vrlo zahtevnih ptica tokom produžene neprekidne nosivosti. Diskutuje se o strategiji ishrane, potrebama i preporukama, dinamici ishrane tokom dana i tokom proizvodnog ciklusa, novim tehnologijama i tehnikama ishrane. Rad ima za cilj da doprinese rešavanju izazova koji se očekuju širom primenom duže neprekidne eksploatacije nosilja genetički sposobnih za to. Maksimalni proizvodni rezultati dostižni su preciznom ishranom namenski formulisanim obrocima, kao i što dužim očuvanjem zdravlja nosilja i kvaliteta ljuske jaja pravilnom ishranom. Preduslov za to je bliska saradnja odgajivača životinja, nutricioniste i proizvođača hrane.

Ključne reči: kokoši nosilje, maksimalna nosivost u jednom ciklusu, tehnologija ishrane, tehnike hranjenja

Acknowledgment

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INNOVATION IN MEAT PACKAGING

Tanja Petrović¹, Snežana Stevanović¹, Dragana Paunović¹, Jasmina Rajić², Viktor Nedović¹

¹University of Belgrade, Faculty of Agriculture, Institute of Food Technology and Biochemistry
Nemanjina 6, 11080 Zemun-Belgrade, Serbia

²Higher Engineering School-Tehnikum Taurunum, Nade Dimić, 4, 11080, Zemun-Belgrade, Serbia

Corresponding author: Tanja Petrović, tpetrovic@agrif.bg.ac.rs

Invited paper

Abstract: Consumer demands for fresh meat are constantly increasing due to its significant nutritional and biological properties. Meat is a rich source of biologically valuable proteins, essential amino acids, vitamins and bio available minerals. However, it is highly perishable and should be packed using appropriate packaging materials and packaging technologies, in order to retain its high quality.

Therefore, this paper will give an overview of innovative techniques of packaging of fresh meat, that satisfy the criteria necessary to prolong shelf life and monitor its quality during distribution and storage, until consumption. In this regard, the application of vacuum packaging, modified atmosphere packaging (MAP), as well as active and intelligent packaging will be reviewed.

Key words: meat, vacuum packaging, modified atmosphere packaging (MAP), active and intelligent packaging

Introduction

It has been evaluated that 99.8% of all food and beverage products are enclosed or enveloped with some packaging materials (*Restuccia et al., 2010*). Development in food packaging are mainly focused on better protection of food products, greater reliability, increase functionality and shelf life and better ecological characteristics of packaging materials and packaging. Global trends such as increasing industrial processing of foods, increasing import and export, less time for preparing meals at home and strict requirements related to food quality and safety have been the main driving force for the packaging industry to explore innovative solutions (*Petrović et al., 2012; Petrović, 2015*).

Packaging plays a significant role in protection of food from physical, chemical and microbiological contamination, provides visibility and displays label information (*Marsh and Bugusu, 2007; Petrović, 2011*). Fresh meat has high water

activity and is a rich source of biologically valuable proteins, essential amino acids, B vitamins, as well as vitamins D and A, folic acid and bio available minerals, especially zinc, iron and phosphorus (Olaoye, 2011). Therefore, it is highly perishable and suitable medium for spoilage due to microbial growth and oxidative reactions. In order to avoid deterioration, reduce weight loss and preserve bright red color it is necessary to use packaging materials with suitable barrier properties to gases, humidity, light and microorganisms (Petrović and Rajić, 2016).

Fresh meat cuts are commonly found in the retail markets in foam-trays with absorbent pad, overwrapped with clear permeable polymer film, which cannot prevent the natural degradation, due to the presence of atmospheric air in the package. However, there are several methods used to prolong the shelf life of fresh meat such as, vacuum packaging, which removes atmospheric oxygen preventing the microbial growth and consequent spoilage of food product (Schmid *et al.*, 2016; Mathew and Jaganathan, 2017); modified atmosphere packaging that involve application of specific gases to maintain product preservation (Floros and Matsos, 2005) as well as active and intelligent packaging which ensure the active participation of the packaging in preserving the quality of meat, providing information on the product quality during distribution and storage until consumption (Pavelková, 2012; Vilela *et al.*, 2018; Lazić *et al.*, 2008). Therefore, the objective of this paper was to give an overview of these innovative methods for fresh meat packaging.

Vacuum and modified atmosphere packaging of fresh meat

Vacuum packaging (VP) is considered as the first commercially developed form of modified atmosphere packaging (MAP), in which air is removed to prevent the growth of spoilage microorganisms, oxidation and discoloration (Stasiewicz, *et al.*, 2014; Schmid *et al.*, 2016). This technique can prolong the shelf life of food by several weeks, at refrigerated storage. The packaging of meat in vacuum has the advantage as a simple technique, but the compression it causes can decrease its original shape and/or increase meat exudate (Church, 1994). VP is typically used for lightly pigmented cuts of pork and chicken, whereas this technique is not suitable for the retail market of fresh beef, because oxygen depletion associated with the low permeability of the packaging material to oxygen can change the meat color. Actually, the conversion of meat pigment deoxymyoglobin to metmyoglobin causes the transformation of meat color from purple-red to brown, which is often rejected by consumers (Jayasingh *et al.*, 2001). To prevent browning of meat the oxygen levels in the package must be less than 0.15 % (Mathew and Jaganathan, 2017). The new approaches in vacuum packaging technology have been the introduction of vacuum skin packaging (VSP), developed to retail small portions of

fresh meat, minced meat, or meat preparations (Stella *et al.*, 2018). VSP packaging wraps firmly around the products, acting as a second skin while removing all atmospheric air from the package. An added value of VSP packaging is the attractive appearance of the meat products, where glossy skin contributed to the presentation of the products to the consumers (Figure1).



Figure 1. Vacuum skin packaging (Source: www.xkpack.com)

In addition, modified atmosphere packaging (MAP) appears as an alternative in preserving the freshness of processed meat with a promising role in the market, as a non-thermal food preservation technique. MAP is a technique used to extend the shelf life of fresh, minimally processed and ready-to-eat foods. In this package, atmospheric air (78% nitrogen, 21% oxygen and 0.03% carbon dioxide), surrounding the food is replaced with a protective gas or selective mixtures of gases in order to reduce undesirable physiological, chemical/biochemical and physical changes and control of microbial growth (Sørheim *et al.*, 1997). The most common used gases in MAP are nitrogen, carbon dioxide and oxygen, combined in certain ratio depending on the product need. Some other gases like carbon monoxide, argon, helium, nitrous oxide and sulfur dioxide should be used for specified food in MAP (Robertson, 2006). In the selection of an appropriate gas atmosphere, water content, water activity, chemical composition, acidity (pH) and sensitivity of the product for specific microbial, chemical and enzymatic degradation should be well considered.

Each gas plays a specific role in extending the product's shelf life and maintaining its sensory and nutritional characteristics. Nitrogen, N₂ (E-941) is tasteless, chemically inert and used as a gas filler to prevent collapse of packaging. Indirectly it prevents growth of aerobic microorganisms and delay oxidation. This gas has low solubility in water and lipids and lower permeability than CO₂/O₂. Oxygen, O₂ (E-948) can cause oxidation of lipids, pigments and vitamins in foods. It accelerates ripening and senescence of fruits and vegetables, changes in color and spoilage caused by the growth of aerobic bacteria and inhibits the growth of strict anaerobes (Church, 1994). However, its low concentration is necessary in MAP for the fresh fruits and vegetables to allow basic aerobic respiration while in

fresh meat it maintains bright red color due to the formation of oxymyoglobin (Floros and Matsos, 2005). Carbon dioxide, CO₂ (E-290) is very soluble in water and fat and possesses bacteriostatic and fungistatic effect (Gill, 1988). It is a colorless gas with a slight odor at very high concentrations (Church and Parson, 1995). The inhibitory activity of CO₂ increases if the storage temperature decreases due to its greater solubility in water and fats, at low temperatures. Molds and yeasts are relatively resistant to carbon dioxide; their growth is suppressed when the carbon dioxide content of the gas mixture is at least 15% (Dixon and Dkell, 1989). In addition, it is possible to control growth of many aerobics, including *Pseudomonas* spp., *Acenatobacter* spp. and *Moraxella* spp., however, high concentrations may stimulate the growth of *Clostridium botulinum* (Farber, 1991; Paramithiotis et al., 2009). The effectiveness of CO₂ also depends on the growth phase of the microorganism. Carbon dioxide increases the duration of the adaptation phase and reduces the growth rate during the logarithmic phase. Carbon monoxide (CO) can also been used in small concentrations in MAP, with high concentrations of CO₂ and absence of O₂, because it retains the bright red color of fresh meat by formation of carboxymyoglobin, (Jeong and Claus, 2011). Although this technology has been allowed in the United States since 2004, the use of CO in MAP is not permitted in the European countries (EU, 1995) due to its toxicity, regardless studies show it pose no risk to consumers when it is used in concentrations up to 1% (Luño et al., 2000; Jeong and Claus, 2011; Fraqueza and Barreto, 2011).

Modification of the gas atmosphere inside the package can be achieved by active or passive manner. Passive modification, in the case of fruits and vegetables, occurs as a result of product respiration or metabolism of microorganisms associated with food (Petrović et al., 2019; Stevanović et al., 2019). The packaging structure is usually consists of polymeric film and the gas permeability through the film also influences the composition of the evolving atmosphere (Robertson, 2006). Active modification involves two different techniques: flushing gas (mechanical replacement of the air) and vacuum compensated. In the flushing technique, the gas is continuously introduced into the package suppressing the air in the packaging, and the package is closed usually by thermoforming. Compensated vacuum technique is performed in two steps; the product passes through a tray and the air is removed using vacuum pump, thereafter the vacuum is disrupted by the desired gas or mixture of gases and the package is heat sealed. The advantage of the latter method is its greater efficiency in removing O₂ at residual levels, below 1% (Smith et al., 1990). In the flushing gas technique, 2-5% residual O₂ remains in packaging, therefore this technique is not suitable for the foods that are very sensitive to oxygen. However, the biggest advantage of the flushing gas process is higher velocity as the operation is continuous (Jovanović and Džunuzović, 2011).

Packaging materials used in MAP are crucial for the success of packaging in a modified atmosphere. The combinations of different polymer materials are select in order to achieve the desired mechanical strength, barrier characteristics (low water vapor transmission and high gas barrier capability or selective permeability for gases) and high sealing ability. The most common materials used are low-density polyethylene (PE-LD), high-density polyethylene (PE-HD), oriented-polypropylene (OPP), polyamide (PA), polyesters (PET), poly vinyl chloride PVC, poly vinylidene chloride (PVdC), polystyrene (PS), ethylene-vinyl acetate (EVAC) and ethylene-vinyl alcohol (EVA) produced as monolayers, multilayers, laminated or metallized films and trays (*Marsh and Bugusu, 2007*). In addition, the permeability of polymer materials are reduced by lowering the temperature, ie under these conditions they have better barrier properties for all gases (*Jovanović and Džunović, 2011*).

Main quality characteristics for fresh meat are its color and microbiological condition. The oxidation state of the muscle tissue and relative proportions of the three myoglobin forms deoxymyoglobin (Mb), oxymyoglobin (MbO) and metmyoglobin (MetMb) affect the color of fresh meat (Figure 2).

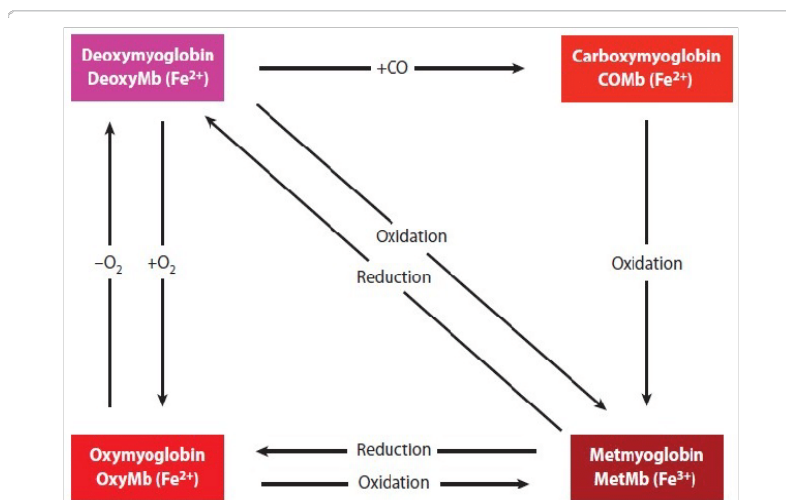


Figure 2. Oxidation state of myoglobin pigment (Source: *Hammad et al., 2017*)

Metmyoglobin is formed primarily in the presence of low oxygen partial pressures, in particular a few millimeters below the surface of the meat. To prevent the formation of this brown layer, a high oxygen pressure is needed in order to form oxymyoglobin, resulting in bright red coloration of meat, which consumers associate with freshness and quality (*Surendranath and Poulson, 2013*). Therefore,

the most commonly used MAP system for fresh meat packaging employs high concentrations of O₂ in combination with CO₂ to suppress the growth of aerobic bacteria, usually 60-80% O₂ and 20-40% CO₂ (Eilert, 2005). Such high oxygen levels can cause some oxidative changes in the meat (lack of meat aroma, rancidity, off-flavor and odor, lack of juiciness, etc.), resulting in sensory deviations (Clausen *et al.*, 2009). According to Zakrys-Waliwander *et al.* (2011), significantly lower oxidative changes could be obtained for beef steaks and ground beef by reducing the oxygen level in MAP to about 50%. In addition, Stahlke *et al.* (2018) pointed out that high oxygen MAP packaging has not been recommended for meat from 8-month-old lamb, because it favored lipid oxidation, psychotropic bacteria growth and decreased the red meat color during storage.

Rosa (2009) studied three gaseous compositions (75% O₂ + 25% CO₂; 50% O₂ + 50% CO₂ and 100% CO₂) and concluded that from a microbiological point of view they kept pork loin steaks for up to 15 days. However, only atmospheres with high O₂ concentrations (> 50%) preserved the color and general appearance of the steaks. On the other hand, oxygen is undesirable for processed meat, such as sausages, bolognese, hams and sliced products. The presence of oxygen in these products influences the color and promotes the growth of many food spoiling microorganisms and also causes the oxidation of lipids, pigments and vitamins (Gokoglu *et al.*, 2010).

A high level of oxygen is also used for poultry without skin in order to retain the color, regardless some investigation pointed out that oxygen should be avoided in poultry and turkey meat, because it may promote an adverse effect due to the development of off-flavor and discoloration caused by lipid (higher proportion of unsaturated fatty acids) and protein oxidation and promote premature browning during cooking (Cornforth and Hunt, 2008; Demirhan and Candogan, 2017). The results of Abdullah *et al.* (2017) have showed that oxygen-free modified atmosphere was preferable for the packaging of organic chicken meat. In addition a negative effect of residual level of oxygen in this low-oxygen MAP may be overcome by using oxygen-scavenging technologies which provide oxidative stability of raw and cooked poultry products (Demirhan and Candogan, 2017).

Fish and sea foods are very sensitive to microbial growth due to their neutral pH and rich nutritional compounds. Selection of gas composition for fish packaging depends of its fatty acids content. Principally, atmosphere of 30% O₂, 40% CO₂ and 30% N₂ has been used for lean fish (Flores and Matsos, 2005). A high level of O₂ prolongs commercial validity of marine fish by reducing trimethylamine oxide, which is mainly responsible for the unpleasant odor of fish, in trimethylamine, (Boskou, and Debevere, 1998). However, in the case of fatty and smoked fish oxygen must be avoided in order to prevent oxidative reaction and development of

rancidity, therefore, gas mixtures compose of 60% CO₂ and 40% N₂ is recommended (Flores and Matsos, 2005).

Active packaging of fresh meat

Active Packaging (AP) changes the conditions of packaged food in order to increase the shelf life, improve safety and sensory properties, while the quality of the packaged product remains unchanged (Kerry *et al.*, 2006). The active compounds from AP may prevent or stop chemical and enzymatic reactions, oxidative degradation and microbial contamination of food. They can also control weight loss, retain the color and integrity of food products during storage. AP techniques include physical, chemical or biological reactions of the active component of AP with an atmosphere above the packaged product, in order to achieve the desired preservation effects (Prasad and Kochhar, 2014). AP functions may be provided by integrating the active substances into the packaging material (usually polymers) or the active substances may be introduced in a separate container in the form of small paper sachets or absorbent pads, before closing the packaging (Vilela *et al.*, 2018). AP techniques involve the use of active absorption systems, active releasing systems as well as controlled release systems – antimicrobials (Otoni *et al.*, 2016).

Meat, poultry, seafood and processed foods are dominant area of AP applications, while oxygen scavengers, which removes residual oxygen after MAP or vacuum packaging, are commonly form of AP (Ahmed *et al.*, 2017). Oxygen absorbers are used in three different concepts; sachets, adhesive labels or active formula are incorporate into the polymer matrix during manufacturing (Figure 3).

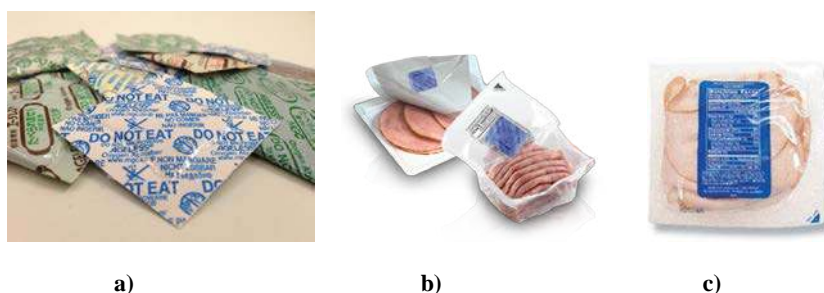


Figure 3. a) Oxygen sachets b) Oxygen absorbers in the form of labels c) Oxygen absorbing substances incorporated into the polymer matrix

(Sources: www.healthcarepackaging.com; www.multisorb.com; www.ital.agricultura.sp.gov.br)

Moisture absorbers remove moisture, preventing the appearance of condensation, indirectly affecting microbial growth. In addition, carbon dioxide emitters are used in MAP for fresh meat cuts, poultry and fish (Nassu *et al.*, 2010). In the contact with product exudate, carbon dioxide is released, leading to the formation of carbonic acid, which acidified the food and reduce microbial growth (Figure 4). In addition, regeneration of CO₂ gas in the package prevents MAP from collapsing.

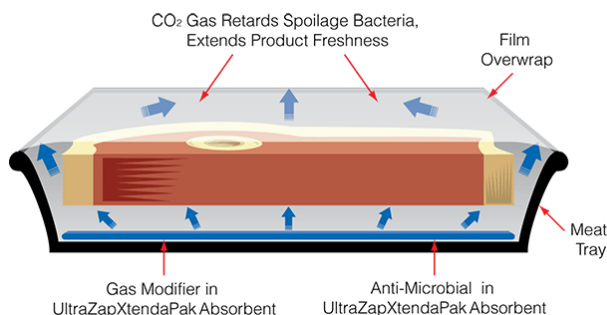


Figure 4. AP technology for carbon dioxide emitter (Source: www.genuineideas.com)

Flavor/odor absorbers may be applied in AP (place at the bottom of trays or incorporate into plastic films) for fresh meat, poultry and fish foods, as they are useful in removing off-odors and flavors generated as a result of protein degradation or lipids oxidation (Prasad and Kochhar, 2014). However, the commercial application of these absorbers is still controversial due to the possibility that they can mask natural spoilage of food, leading to the misjudgment of the product quality by the consumers, during purchase and consumption.

The promising application of AP is antimicrobial packaging, which are based on the application of different active substances like: chlorine dioxide, silver, silver zeolite, triclosan, glucose oxidase, ethanol vapor emitting, natamycin, sulphur dioxide and allyl isothiocyanate (Vilela *et al.*, 2018). In addition, one of the prominent aspects of antimicrobial packaging is the addition of antimicrobial substances as well as essential oils and plant extracts into the biodegradable packaging materials as sustainable and eco-friendly materials which reduces waste (Dawson *et al.*, 2008; Maisanaba *et al.*, 2017).

Meat and fish with high lipid content may be also packed using antioxidant agents in AP (Sun and Holley, 2012). This packaging may prevent oxidation reactions in fresh meat, which are associated with degradation of essential fatty acids, proteins and lipid soluble vitamins, formation of off-flavors and odors, oxidation of pigments and meat discoloration (Tian *et al.*, 2013; Ganiari *et al.*, 2017).

Intelligent packaging of fresh meat

It is estimated that 30% of the food produced for human consumption is lost or wasted along the food supply chain (FAO, 2015). It is anticipated that the world's population will attain 9.6 billion by 2050 and this will require an increase of 70% in food availability (Rezaei and Liu, 2017). Therefore the introduction of intelligent packaging (IP) can be useful tool to provide real-time status of food quality, thus eliminate the rejection of food products with satisfactory quality attributes. Intelligent packaging (IP) is designed to monitor the quality of packaged foods in order to provide information during their transport and storage until consumption (Ghaani *et al.*, 2016). An IP system contains indicators, sensors or RFID tags, which are small, inexpensive and capable to observe and monitor internal and/or external changes in the product surroundings. The most common used IP system in meat and fish packaging is oxygen sensors, and/or biosensors, seal and leak indicators, time temperature indicators, freshness and pathogen indicators and radio frequency tags (Pavelková, 2012).

Oxygen sensor and seal and leak indicators (O_2 and CO_2) can be applied to detect food quality, or to prove the effectiveness of oxygen absorbers (Fuentes *et al.* 2016). The principle of leak indicators is based on color changes of indicator as a result of chemical or enzymatic reactions in meat products. In addition, the application of time temperature indicator (TTI) enable to permanently monitor the temperature in refrigerated and frozen products, along the food supply chain (Wanihsuksombat *et al.*, 2010). During storage of food product changes in the quality can also occur as a result of microbial growth, therefore the presents of metabolites like organic acids, carbon dioxide, aldehydes, alcohols (ethanol) biogenic amines, volatile nitrogen compounds such as ammonia or sulfur compounds can be used as freshness indicators (Arvanitoyannis and Stratakos, 2012). Different indicators which can be used in packaging of meat and fish are presented in the figure 5.

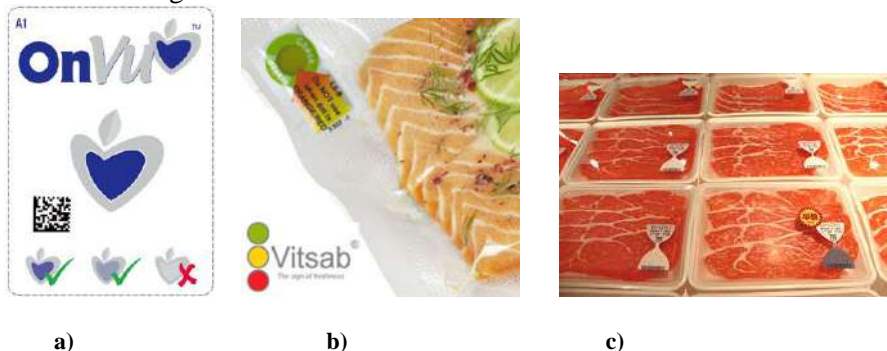


Figure 5. Examples of different indicators used in packaging of meat

- a) OnVu TTI - activated by UV light; center of TTI become a dark blue, producing a clear contrast with the light-colored edge which is the reference color. According to the temperature history of the product indicator started to fade (Source: www.ifworldesignguide.com);
- b) Fresh-Check indicator (www.fresh-check.com);
- c) Freshness indicator - label changes the color by reacting with the ammonia produced during the degradation of meat products - bar cod becomes unreadable to scanning and selling the product (Source: www.interempres.net).

RFID technology does not belong either to sensor or indicator but represents separate electronic data based form of IP. The possibility of RFID application in the food industry can enable easier monitoring of product traceability, which may improve the efficiency of the food supply chain (Mack *et al.*, 2014; Majid *et al.*, 2018). When food product is packed, the RFID microchip is put on the packaging or pallet while all the necessary information about the food products and appropriate programs are entered into the chip. A sensor in the microchip provides real-time information, such as temperature, moisture, gas composition in MAP, nutritional information, cooking instructions etc. (Eom *et al.*, 2014; Fuertes *et al.*, 2016). These data can be further transferred to the computer via antenna, by using reader which emits radio waves (Figure 6).

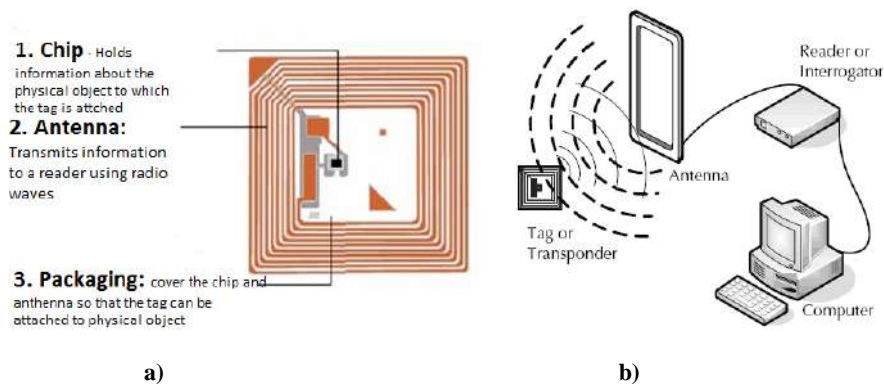


Figure 6. a) Radio frequency identification chip; b) RFID system
(Sources: www.cxjrfidfactory.com (adapted); www.epc-rfid.info)

Conclusion

Meat is a highly perishable food because of its chemical composition and biological characteristics. In order to avoid deterioration and offer safe and quality products to the consumers, meat industry must use appropriate packaging solutions. The application of vacuum and modified atmosphere packaging can markedly

prolong the shelf life. In addition, active packaging can also enhanced protection to the packed meat compared to conventional packaging, while intelligent packaging can monitor the condition of the meat, making the information flow more efficiently within the food supply chain.

Further development of new packaging solutions will continue to be consumer oriented and in the future we should expected the application of more interactive packaging, greater use of active and intelligent packaging as well as greater concern about environmental aspect of packaging.

Inovacije u pakovanju mesa

Tanja Petrović, Snežana Stevanović, Dragana Paunović, Jasmina Rajić, Viktor Nedović

Rezime

Potražnja za svežim mesom neprestano raste od strane potrošača, zbog njegovih značajnih nutritivnih i bioloških svojstava. Meso je bogat izvor biološki vrednih proteina, esencijalnih amino kiselina, vitamina i biosvojivih minerala. Međutim, meso je lakokvarljiv proizvod pa ga je neophodno pakovati primenom odgovarajućih ambalažnih materijala i tehnologija pakovanja, kako bi se zadržao njegov visok kvalitet.

Radi toga će ovaj rad dati pregled inovativnih tehnika pakovanja svežeg mesa, koje zadovoljavaju kriterijume potrebne za produženje roka trajanja, kao i praćenje njegovog kvaliteta tokom distribucije i skladištenja, sve do njegove upotrebe. Sa tog stanovišta, biće dat prikaz vakumskog pakovanja, pakovanja uz primenu modifikovane atmosfere gasova, kao i aktivnog i inteligentnog pakovanja.

Ključne reči: meso, vakuum pakovanje, pakovanje u modifikovanoj atmosferi gasova, aktivno i inteligentno pakovanje

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NITROGEN STATUS EVALUATION OF GRASS-LEGUME SWARDS UNDER FOUR N FERTILIZATION LEVELS

Zorica Bijelić¹, Violeta Mandić¹, Vesna Krnjaja¹, Dragana Ružić-Muslić¹, Aleksandar Simić², Zdenka Škrbić¹, Dušica Ostojić Andrić¹

¹Institute for Animal Husbandry, Belgrade-Zemun, Republic of Serbia

²University in Belgrade, Faculty of Agriculture, Belgrade-Zemun, Republic of Serbia

Corresponding author: Zorica Bijelić, zonesh@gmail.com

Invited paper

Abstract: Forage grass-legume mixture are highly productive ambience of different plant species intended for animal nutrition. The competitiveness of species in the mixtures is one of the important traits that significantly affect grassland management. The goal of the study was to evaluate nitrogen nutrition index of pure lucerne and their mixtures with grasses and sainfoin subjected to four different levels of nitrogen in three years duration. The experiment had a randomised block design with four replicates and eight treatments. Examined treatments were pure lucerne crop and mixtures of lucerne, orchard grass, tall fescue and sainfoin in the same proportion and four nitrogen fertilization rates (0, 70, 140, 210 kg ha⁻¹). Pure lucerne achieved higher yield by 12.8% than their mixtures. Forage production was the highest at 210 kgN ha⁻¹ which was 14.6% more than treatments without nitrogen. Nitrogen uptake by the plant that were fertilized with N was significantly higher than the plants that were not fertilized. In all three experimental years control nitrogen treatment had satisfactory value of nitrogen nutrition index while other N treatments showed luxury consumption. Soil N reserves and nitrogen fixation in the treatment where N was not applied, were sufficient to ensure enough biomass production.

Key words: biomass production, grass-legume mixtures, nitrogen consumption

Introduction

In addition to water, nitrogen is one of the major limiting factors in production of forage biomass in the natural ecosystems. After mineralization of organic matter in the soil, N becomes available to plants in the form of nitrate and ammonium ions. The amount of available nitrogen in the soil varies from year to

year, depending on a number of factors such as: climate, mineralization intensity and amount of organic matter. How much nitrogen will be used from the soil depends on plant species and of its yield potential. Different species in the grass-legumes mixtures have different capabilities for nitrogen accumulation and production of biomass, therefore, N assimilation in grass-leguminous mixtures strongly depends on the selection of species for mixtures. Plant requirements for nutrients are not constant during vegetative growth. After defoliation in the early stages of regrowth, larger quantities of N for the development of leaf mass and photosynthesis are required (*Marino et al., 2004*). Further, the structural composition of plant tissues is dominated by components with low nitrogen contents such as cell walls, which means that the N requirements per unit of dry matter decrease (*Lemaire and Salette, 1984*).

Nitrogen originating from the soil and mostly deriving from mineralization is often not enough for plants to achieve potential yield. Grass-leguminous mixtures also provide N from symbiotic nitrogen fixation where the legumes fix the atmospheric N₂ and create for themselves as well as associated species, conditions with larger N supply. In that way, legumes and grasses meet their own N-demand deriving from symbiosis more than 80% or 40% of N, respectively (*Nyfelers et al., 2011*). The remaining part of the missing nitrogen is recovered from soil nitrogen and N from mineral fertilizers. If the amount of added nitrogen is not fully utilized for yield production, it leads to an increased risk of nitrogen leaching into deeper soil layers, which is a major problem for the environment (*Tripolskaja and Verbyliene, 2014, Marin et al., 2017*). In order to avoid these losses, the French have developed a mathematical model for diagnosis of N status during the vegetative cycle of plants that could be further used for N fertilization planning. This model is based on a critical concentration of nitrogen (N_c) in plants, i.e. a minimum amount of N necessary to achieve maximum yield (*Lemaire and Gastal, 2009*). According to *Lemaire and Gastal (1997)* the equation for calculating N_c for perennial grasses and legumes is:

$$N_c = 4.8DM^{-0.32}$$

The content of nitrogen in plants during vegetation is not constant and it changes depending on the vegetative growth of the plant. *Lemaire et al. (1985)* showed that for grasses and lucerne the decline in plant N concentration (%N) is related to dry matter accumulation (DM) whatever the climatic or the species and genotype. Also, *Farruggia et al. (2004)* have concluded that values of N_c at the start of the growing period are high and decline during growth, in relation to dry matter accumulation.

Nitrogen nutrition index (NNI) is defined as the ratio between the actual concentration of nitrogen in the plants and N_c (Lemaire and Meynard, 1997):

$$NNI = \frac{N_m}{N_c}$$

According to Duru *et al.* (1997), when NNI is close or equal to 1 (0.8-1), the N plant status is considered satisfactory. A deviation from this range, when the NNI is below 0.8, means that there is insufficient supply of N, or when NNI is above 1 there is an oversupply of N: a value of 0.6 indicating that crop N availability was only 60% of the critical level (Louarn *et al.*, 2015).

Today, due to increasing pollution of natural resources, water, air and soil, scientists place the environment protection as the priority, which undoubtedly limits agricultural production. Also, taking into account climate change and increased energy consumption for the production of mineral fertilizers, agriculture carries the global burden of humanity, whether to increase production in order to feed the growing human population or reduce the impact on the environment and warming of the atmosphere. For these reasons, the monitoring of the dynamics of nitrogen adoption during the vegetative cycle of plants and the assessment of the nutrition status is of great importance.

The aim of this study is to evaluate the nitrogen nutrition index of different grass-legume mixtures fertilized with four different levels of nitrogen and to predict their nutritional status through mathematical model.

Materials and Methods

The study was carried out on the experimental field of Institute for Animal Husbandry, Zemun, Belgrade (44°49'N, 20°17'E, and elevation 96 masl), during three year period. At the experimental site, the mean annual precipitation was 714.4 mm and mean annual temperature 16.7°C. The soil is a low carbonate chernozem with pH of 7.26 and well supplied with humus and nitrogen. Agrochemical characteristics of the topsoil arable (0–20 cm) layer, before the experiment, were: CaCO_3 – 0.33%, humus – 4.35%, total N- 1975 ppm, P_2O_5 - 90.9 mg 100⁻¹, K_2O - 16.2 mg 100⁻¹.

The study plot was previously planted with maize. The experiment was arranged in a completely randomized block design with four replicates. The net plot size was 2 × 5 m. Sowing was done in the early spring after land preparation. Lucerne was sown in monoculture and in mixtures with grasses and sainfoin in a different ratio: lucerne 50% + orchardgrass 50%, lucerne 33.3% + orchardgrass 33.3% + tall fescue 33.3%, lucerne 25% + orchardgrass 25% + tall fescue 25% + sainfoin 25%. Four different nitrogen treatments of 0, 70, 140 and 210 kg ha⁻¹ were applied, one

half, early in the spring at the beginning of vegetation and the second half, after the first cut. The nitrogen source used was ammonium nitrate (AN) with a N concentration of 34%.

The swards were cut 4 times per year. In three year period, aboveground fresh biomass was measured by cutting the sward in each plot with a beginning of lucerne flowering at approximately 5 cm above ground. Samples of 1 kg were then randomly taken from the cut material and dried in the oven at 60°C for 72 h to determine the dry matter content and the total yield. These samples were used for analyses of N content by using the Kjeldahl method.

The critical N content (N_c) was estimated by applying mathematical model developed by *Lemaire and Gastal (1997)*, for temperate grasses and lucerne, while nitrogen nutrition index was calculated as the ratio between the real concentration of N in the plants and N_c , according to *Lemaire and Meynard (1997)*.

Statistical analyses were performed using *Statistica 8 (2007)*. Analyses of variance (ANOVA) were used to test the effects of categorical factors on tested crop properties while differences between treatments means were estimated by the LSD test. The response of NNI to other examined properties, differed among nitrogen levels, is shown graphically on the scatterplot.

Results and Discussion

Total dry matter production was significantly higher for pure lucerne sward and fertilized treatment. In the first study year lucerne mixture with orchardgrass achieved the highest yield compared to pure lucerne crop and other lucerne mixtures. In further years, the yield of pure lucerne crop was predominant. Our results are in agreement with *Foster et al. (2014)* whose monoculture lucerne crop shows higher DMY than most grass-lucerne mixtures. On the other hand, there are studies where the lucerne mixtures have achieved higher yields than pure lucerne crop (*Malhi et al., 2002*), as well as study which proves that increasing the number of species in the mixture increases the yield (*Papadopoulos et al., 2012*). In agricultural ecosystems, grass-legume mixtures have the potential to increase productivity, herbage nutritive value and resource efficiency (*Peyraud et al., 2009*). Recent results of a great European experiment, with two grasses and two legumes at thirty-one sites, have demonstrated strong positive mixing effects (*Finn et al., 2012*). These findings are based on the complementary utilization of natural resources such as light, water, or nutrients. However, there are studies showing the presence of species in mixtures that are well adapted to the agronomic environment (soils, climate, and management), highly productive and can have major effects on productivity rather than species richness. So, the relationship between biomass productivity and species richness can vary depending on the presence or absence of

certain species (*Picasso et al.*, 2008). This may have been the case in our study where set of climatic conditions and pedological characteristics have acted more favorably on the development of lucerne compared to the examined grasses.

The forage DM yield of pure lucerne and grass-legume mixtures was significantly affected by N fertilization in all three study year (Table 1). The DM yield increased with increasing N rates. The highest yields were achieved under 210 kgN ha⁻¹ while the lowest without N fertilization. *Tomić et al.* (2011) and *Bijelić et al.* (2017) also state that N fertilization significantly favors the yield of grass-legume mixtures. On the other hand, in some studies, fertilization shows no effect on grass-legume dry matter yields (*Yolcu et al.*, 2010). These differences could be explained by differences in climate, soil conditions, supply of N as species characteristics.

There were no statistically significant differences between the crops in N concentration, but only in the first year of testing. In other years, the pure crop of lucerne had significantly higher N content. Concentration of N was in the range 23.9-26.9 g kg⁻¹ DM.

According to *Fairey* (1991), herbage productivity and quality are more influenced by crop-management factors like harvesting frequency and N fertilizer supply than by the species composition of the seeded mixture. In our research fertilization significantly increased N content in forages in every study year. Also, in the study of *Sartor et al.* (2014), pasture biomass nitrogen content is significantly affected by the addition of nitrogen fertilizers. Addition of 200 and 400 kgN ha⁻¹ increases N content by 29.4% and 35.2% respectively. Compared to this, in our experiment, lower doses of nitrogen resulted in a larger N content increase. So, the level of 210 kgN ha⁻¹ increased on average 58.8% compared to control. Species in grass-legumes mixtures respond differently to N fertilization (*Martin et al.*, 2017). The lack of N response from legumes is due to their N-fixing ability. The N concentration of legumes was unaffected by increasing N-fertiliser rate, whereas in grasses and herbs it increased.

Table 1. Dry matter yield (DM), N concentration and nitrogen nutrition index (NNI) of pure and mutured crop at different level of added N in three years period.

Years	2010			2011			2012		
Factors	DM (tha ⁻¹)	N (gkg ⁻¹ DM)	NNI	DM (t ha ⁻¹)	N (gkg ⁻¹ DM)	NNI	DM (tha ⁻¹)	N (gkg ⁻¹ DM)	NNI
Mixtures									
M	15.6 ^a	25.1	1.25	16.0 ^a	25.7 ^a	1.28	16.4 ^a	26.9 ^a	1.31
I	16.1 ^a	24.2	1.21	14.1 ^c	24.8 ^b	1.21	14.1 ^{bc}	25.2 ^c	1.24
II	14.7 ^b	23.9	1.19	14.2 ^c	24.1 ^b	1.19	14.2 ^b	25.7 ^{bc}	1.28
III	14.6 ^b	24.3	1.19	14.8 ^b	24.9 ^b	1.23	13.3 ^c	26.6 ^{ab}	1.29
F Prob.	**	NS	NS	**	**	NS	**	*	NS
N fertilization									
0	14.8 ^b	17.3 ^c	0.88 ^c	13.3 ^d	17.9 ^d	0.87 ^d	13.3 ^c	19.6 ^c	0.95 ^c
70	15.3 ^b	25.7 ^b	1.26 ^b	14.2 ^c	24.5 ^c	1.19 ^c	14.9 ^b	26.0 ^b	1.26 ^b
140	14.7 ^b	26.9 ^a	1.32 ^{ab}	15.0 ^b	27.2 ^b	1.34 ^b	14.1 ^b	29.5 ^a	1.41 ^a
210	16.1 ^a	27.6 ^a	1.39 ^a	16.5 ^a	29.8 ^a	1.51 ^a	15.7 ^a	29.5 ^a	1.51 ^a
F Prob.	**	**	**	**	**	**	**	**	**
Interaction of two factors									
F Prob.	**	NS	NS	**	NS	NS	*	NS	NS

M-pure lucerne, I-lucerne+orchardgrass, II-lucerne+orchardgrass+tall fescue, III-lucerne+orchardgrass+tall fescue+ sainfoin

* F statistic significant at the 0.05 probability level,

** F statistic significant at the 0.01 probability level,

NS, means nonsignificant.

Type of mixtures showed no significant impact of NNI values of crops. Pure lucerne sward had higher values of NNI compared to its mixtures. Also mixtures with high contribution of legumes had greater values of NNI. This fact could be attributed to nitrogen fixation. Also, *Razec and Razec (2006)*, show in their research that values of NNI increase with the increase of legume content in the mixture. Lucerne like other examined legumes has great positive influence on the improvement of sward N supply (*Kadžiuliene and Kadžiulis, 2007*). However, lucerne-based swards without N fertilization reach indices close to 1.0.

Nitrogen nutrition index of grass-legumes mixtures were generally significantly influenced by N fertilization across all investigation years. Values of the NNI from 0.8 to equal to 1.0 indicate that the crop is in the situation of unlimited N supply. Treatment without N fertilization had NNI values from 0.88-0.95 which is considered optimum for supply of N. After successive nitrogen application from 70-210 kgN ha⁻¹ lucerne and their mixtures start to show luxury consumption (Figure 1.).

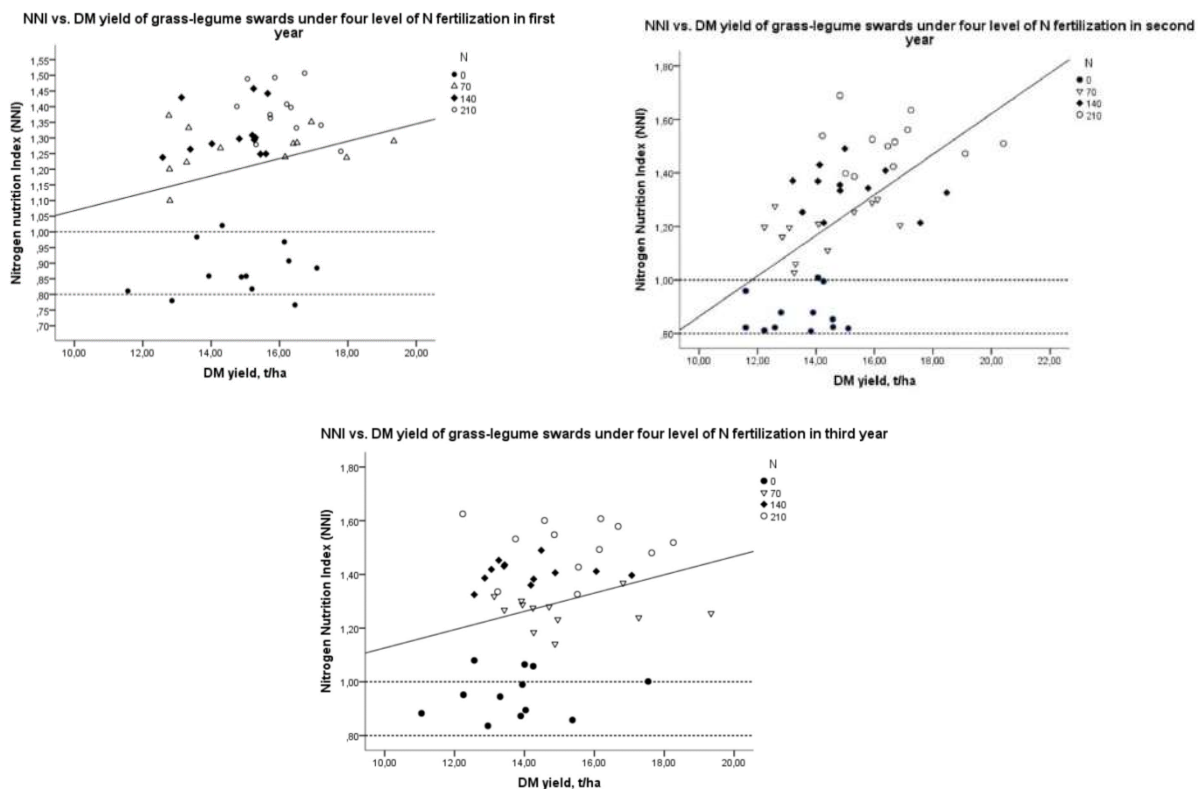


Figure 1. NNI vs. DM yield of grass-legume swards under four different levels of N fertilization in three years period.

This situation showed that the swards were grown in a good soil conditions of nitrogen supply which together with the nitrogen fixation provides optimal environment for growth and development of plants. Also, applied fertilizer can contribute to accelerating the process of decomposition of organic matter, the release of nutrients and the increase of their availability for the plants. Similar results is reported by *Sartor et al. (2014)* where natural pasture of *Urochloa plantaginea* under fertilization with two N levels of 200 and 400 kg ha⁻¹, shows consumption beyond what would be needed for its growth. On the other hand, 50 years old sward, with dominant species of *Agrostis* spp., *Holcus lanatus* and *Lolium perenne*, have not identified excessive values of the NNI for any fertilization level (*Farrugia et al., 2004*). The NNI observed for the 120 and 60 kgN ha⁻¹ treatments were between 1.0 and 0.8.

Conclusion

In given agroecological conditions, the pure lucerne and the two-component mixture of alfalfa and orchardgrass yielded more than the other lucerne swards. Also, pure lucerne crop had significantly higher N content in relation to its mixtures.

N fertilization significantly increased DM yield of pure lucerne and grass-legume mixtures as well as the content of N in plants.

In grasslands, NNI has proved to be a useful diagnostic tool of the N status. Pure lucerne sward had higher values of NNI compared to their mixtures. Also mixtures with high contribution of legumes had greater values of NNI. NNI values were significantly influenced by N fertilization. Treatment without N fertilization had NNI values from 0.88-0.95 which indicated that the crop was in the situation of nonlimited N supply. Nitrogen treatment of 70, 140 and 210 kgN ha⁻¹ showed luxury consumption.

We can generally conclude that the sward crops were grown under conditions of good nitrogen supply. Possibly, the addition of a small quantities of N at the initial growth stages would only be justified.

Evaluacija azotnog statusa travno-leguminoznih smeša pod uticajem četiri nivoa đubrenja

Zorica Bijelić, Violeta Mandić, Vesna Krnjaja, Dragana Ružić-Muslić, Aleksandar Simić, Zdenka Škrbić, Dušica Ostojić-Andrić

Rezime

Smeša krmnih trava i mahunarki je visoko produktivna sredina različitih biljnih vrsta namenjenih za ishranu životinja. Konkurentnost vrsta u smešama je jedna od važnih osobina koje značajno utiču na upravljanje travnjacima. Cilj studije bio je da se proceni indeks ishrane azotom čiste lucerke i njenih smeša sa travama i esparzetom pod uticajem četiri različita nivoa azota u trajanju od tri godine. Eksperiment je imao randomizirani blok dizajn sa četiri ponavljanja i osam tretmana. Ispitivani tretmani bili su čist usev lucerke i smeše lucerke, ježevice, visokog vijuka i esparzete u istom odnosu i četiri doze azotnog đubrenja (0, 70, 140, 210 kg ha⁻¹). Čista lucerka je postigla veći prinos za 12,8% u odnosu na njene smeše. Proizvodnja krme bila je najveća sa 210 kg ha⁻¹, što je za 14,6% više od tretmana bez azota. Uzimanje azota od strane biljke koja je đubrena N je bila

značajno viša od biljaka koje nisu bile đubrene. U sve tri eksperimentalne godine kontrola je imala zadovoljavajuću vrednost indeksa ishrane azotom, dok su ostali N tretmani pokazali preteranu potrošnju. Rezerve N u zemljištu i fiksacija azota u tretmanu gde N nije primenjen, bili su dovoljni da osiguraju dovoljnu proizvodnju biomase.

Ključne reči: produkcija biomase, travno-leguminozne smeše, potrošnja azota

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SALINITY STRESS EFFECT ON SEED GERMINATION AND SEEDLING GROWTH OF SOME CROP PLANTS

Violeta Mandić¹, Zorica Bijelić¹, Vesna Krnjaja¹, Maja Petričević¹, Aleksandar Stanojković¹, Marija Gogić¹, Aleksandar Simić²

¹Institute for Animal Husbandry, Autoput 16, 11080, Belgrade-Zemun, Republic of Serbia

²Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080, Belgrade, Republic of Serbia

Corresponding author: Violeta Mandić, violeta_randjelovic@yahoo.com

Invited paper

Abstract: Soil salinity is one of the most important factors limiting crop productivity. It is known that the agricultural soils with high concentrations of salts increase day by day. For this reason, it is imperative to monitor the tolerance of crops and genotypes to salt stress because they have different threshold sensitivity. Generally, higher salinity levels in the media negatively affect the germination of seeds and seedling growth of most crops. Germination, germination speed and seedling growth parameters significantly decrease with increasing salt concentrations in the media. Soil salinity adversely affects germination, resulting in poor plant stand. Therefore, the development of salt tolerant genotypes of crops with promising yields would be an ideal solution for growing plants on these soils, as well as sustainable food and feed production.

Key words: germination, seedling growth, osmotic stress, crop

Introduction

Soil is the most limiting factor for crop production in the worldwide. Therefore, the production of food for humans and feed for animals is limited. The soil salinization is big problem for agricultural productivity worldwide. Generally, crop plants are sensitive to soil salinity. Soil salinity is the abiotic factor that limits the germination and early seedling growth of most crop plants. *Jamil et al. (2011)* estimate that more than 50% of the arable land will be salinized because of low rainfall, high surface evaporation, weathering of native rocks, irrigation with saline water and poor agricultural practices, by 2050. In Serbia, about 4.6% of agricultural soils are saline and alkaline soils. Saline soils are contaminated with 40 mM NaCl salts and have $EC_e > 4 \text{ dS m}^{-1}$ and osmotic potential $< 0.117 \text{ MPa}$ (*Ashraf, 2009*). High levels of soil salinity reduce the ability of plants to absorb water and plant growth (*Munns, 2002*), impair ions absorption (*Karimi et al.,*

2005), increase accumulation of a toxic ions (Nawaz *et al.*, 2010). The salinity negatively affects seed germination, seedling growth and enzyme activity (Seckin *et al.*, 2009), growth, development, yield and quality of plants (Jouyban, 2012). Shahbaz and Ashraf (2013) conclude that soil salinity reduces cultivated land area, productivity and quality of crops. Ashraf (2004) points that the saline soil causes osmotic stress due to low osmotic potential of soil solution, ionic stress because specific toxicity effect on ions, nutritional imbalances or a combination of mentioned factors. Also, Muscolo *et al.* (2013) has proved that the high soil salinity has a strong impact on plants because it causes osmotic stress, oxidative stress, ion toxicity, nutritional disorders, alteration of metabolic processes, membrane disorganization and reduction of cell division and expansion. Mandić *et al.* (2011) conclude that testing of genotypes at early seedling growth would be especially helpful in identification and selection of genotypes for particular soil. In this paper, we characterize the effect of the induced water deficit by NaCl (osmotic stress) on germination and seedling growth of a few crops commonly sowed in areas of Serbia. Testing of genotypes of crops at the early seedling stage under different concentrations of NaCl in the growing medium could be helpful in the identification and selection of genotypes for cultivation on saline soils.

Effect of NaCl-induced osmotic stress on seed germination and seedling growth of maize

The high-quality seeds are essential for successful crop production. However, water availability and movement in the medium are important factors which promote germination, root and shoot elongation. The germination is not possible under conditions of low water potential (Singh *et al.*, 2013). Minimum moisture in the soil is necessary for germination of seeds, i.e. for the restart of embryonic axis growth, the intensification of breathing and other metabolic activities and releasing of energy and nutrients (Carvalho and Nakagawa, 1988). However, high salt content of the soil causes lower osmotic potential in the soil solution than in the seed cells which prevents the absorption of water. Therefore, the key to problem is osmoregulation in the first phase of salt stress. Na toxicity affects the seedling growth in the second phase of salt stress (Schubert *et al.*, 2009). The soil limits germination and early seedling growth, crop growth and productivity (Flowers, 2004).

Maize is moderately sensitive to salinity (Ouda *et al.*, 2008), but the maize genotypes differ in resistance and tolerance to salt (Khodarahmpour, 2012). The maize hybrids vary in their tolerance and phytotoxicity to high soil salinity. Thus, Mandić *et al.* (2014a) have concluded that Serbian maize hybrids cannot tolerate high salt concentrations in the medium, where the hybrid ZP 666 showed better

tolerance than hybrid ZP 560 (Table 1 and 2). These authors find that the germination energy, germination, root length, shoot length, root fresh weight, shoot fresh weight, root dry weight and shoot dry weight of maize seedling decrease significantly with increasing osmotic stress induced by NaCl. Authors have concluded that the germination sensitivity threshold of maize is treatment with -0.3 MPa. The osmotic stress causes loss of turgidity of the cells involved in elongation which inhibits the growth of root.

If salinity levels in the soil increases, seed germination of maize decreases (*Khayatnezhad and Gholamin, 2011; Miroslavljević et al., 2013*). Generally, under salinity conditions, the root elongation is more sensitive than shoot elongation (*Demir and Arif, 2003*). *Leishman and Westoby (1994)* find that maize genotypes with longer root systems have higher resistance to low osmotic stress. Salt stress reduces germination and parameters of maize seedlings due to ion toxicity, osmotic and oxidative stress (*Sozharajan and Natarajan, 2014*). Generally, Na⁺ and Cl⁻ are metabolically toxic to cell (*Taiz and Zeiger, 2002*). Many researches have showed that increased salt concentration in the medium reduces germination rate, germination speed, root and shoot length, germination index, root and shoot dry weight of maize seedling and seedling vigor index (*Carpici et al., 2009; Idikut, 2013*).

Table 1. The effects of hybrid and different osmotic stress on germination energy (GE), germination (G), root length (RL), shoot length (ShL), root fresh weight (RFW), shoot fresh weight (ShFW), root dry weight (RDW) and shoot dry weight (ShDW).

Factor	GE %	G %	RL cm	ShL cm	RFW mg	ShFW mg	RDW mg	ShDW mg
Hybrid (A)								
ZP 560	16.9	63.2	4.2 ^b	1.1 ^b	45.8	32.5 ^b	6.5 ^b	4.2 ^b
ZP 666	15.8	65.3	6.4 ^a	2.1 ^a	45.9	43.9 ^a	7.6 ^a	6.0 ^a
F test	ns	ns	**	**	ns	**	*	**
Osmotic stress, MPa (B)								
0	34.9 ^a	97.2 ^a	12.2 ^a	4.7 ^a	82.3 ^a	135.6 ^a	10.7 ^a	12.5 ^a
-0.3	30.5 ^{ab}	94.0 ^{ab}	7.8 ^b	2.0 ^b	61.8 ^b	48.9 ^b	8.9 ^b	6.9 ^b
-0.6	24.8 ^b	83.0 ^b	5.2 ^c	1.8 ^b	48.3 ^c	15.3 ^c	7.9 ^{bc}	3.9 ^c
-0.9	6.2 ^c	60.8 ^c	3.1 ^d	0.6 ^c	39.4 ^c	13.3 ^c	6.8 ^c	3.0 ^{cd}
-1.2	1.2 ^d	37.8 ^d	2.0 ^e	0.4 ^c	26.4 ^d	10.9 ^c	4.9 ^d	2.6 ^d
-1.5	0.2 ^e	13.0 ^e	1.3 ^e	0.2 ^c	16.9 ^d	5.1 ^c	3.2 ^e	1.5 ^e
F test	**	**	**	**	**	**	**	**
A × B	ns	ns	**	**	ns	**	ns	**

Means followed by the same letter within a row are not significantly different by Duncan's Multiple Range Test at the 5% level; ** - significant at 1% level of probability, * - significant at 5% level of probability and ns - not significant

Table 2. The effects of hybrid and different osmotic stress on rate germination index (RGI), seedling vigor index (SVI), relative seedling water content (RSWC), phytotoxicity of root (PhR), phytotoxicity of shoot (PhSh) and dry matter stress tolerance index (DMSI).

Factor	RGI %		SVI	RSWC %	PhR %	PhSh %	DMSI %
Hybrid (A)							
ZP 560	2.7		457.6 ^b	83.8 ^a	55.4	69.8 ^a	48.9 ^b
ZP 666	3.3		706.6 ^a	81.2 ^b	57.6	62.5 ^b	55.9 ^a
F test	ns		**	*	ns	**	**
Osmotic stress, MPa (B)							
0	10.3 ^a		1642.2 ^a	89.2 ^a	0 ^a	0 ^a	100.0 ^a
-0.3	4.0 ^b		920.4 ^b	85.5 ^a	35.4 ^b	57.6 ^b	68.3 ^b
-0.6	2.2 ^{bc}		583.9 ^c	81.3 ^b	56.8 ^c	65.0 ^b	51.0 ^c
-0.9	0.8 ^c		223.5 ^d	80.9 ^b	74.0 ^d	88.3 ^c	42.1 ^d
-1.2	0.8 ^c		97.9 ^{de}	79.2 ^b	83.6 ^e	91.1 ^c	32.4 ^e
-1.5	0 ^c		24.4 ^e	78.9 ^b	89.4 ^f	94.8 ^c	20.3 ^f
F test	**		**	**	**	**	**
A × B	ns		**	*	ns	**	**

Means followed by the same letter within a row are not significantly different by Duncan's Multiple Range Test at the 5% level; ** - significant at 1% level of probability, * - significant at 5% level of probability and ns - not significant

Effect of NaCl-induced osmotic stress on seed germination and seedling growth of red clover

The legumes, especially red clover, are highly salt-sensitive. *Asci (2011)* concludes that the germination and seedling growth of legume on saline soils determines the degree of crop establishment. Salinity resistance to germination seeds of legume is heritable trait important for the selection of salt-resistant genotypes (*Ashraf et al., 1987*). In general, high salt concentration in the germination medium negatively affects the germination and seedling growth of most legumes, such as Egyptian, red and Persian clovers (*Gravandi, 2013*), strawberry clover (*Can et al., 2013*), white clover and alfalfa (*Zhanwu et al., 2011*), *Medicago ruthenica* (*Guan et al., 2009*) and yellow sweet clover (*Ghaderi-Far et al., 2010*). *Mandić et al. (2014b)* have reported that the Serbian red clover cultivars (Kolubara, K-32, K-17 and K-39) are very sensitive to salt, especially cv. K-32 (Table 3). Authors report that germination, seedling growth and vigor index significantly decrease with increasing salt concentration in the germination medium. In general, salinity lowers osmotic potential, resulting in decreased

availability of water. Increasing salinity levels significantly decrease vigor index, and hence the ability of a seed to produce normal seedlings.

In another study, *Mandić et al. (2019)* have found that the cv. K-17 has higher germination energy, germination and vigor index than cv. K-32 and that the tested parameters have not differed between 0 and 50 mM NaCl. However, increases in salt concentration over 100 mM NaCl significantly reduce these parameters.

Table 3. The effects of cultivar and NaCl concentration level on germination energy (GE), germination (G), percentage of dead or infected seeds (DIS), percentage of hard seed (HS), normal (NS) and abnormal seedlings (AS), root length (RL), shoot length (ShL), fresh (FW) and dry weight of seedling (DW) and seedling vigor index (SVI)

Factor	GE %	G %	DIS %	HS %	NS %	AS %	RL cm	ShL cm	FW g	DW g	SVI
Cultivar (A)											
Kolubara	53.6 ^a	63.6 ^a	36.2 ^b	0.2 ^c	52.4 ^a	11.2	1.5 ^b	4.8	9.15	1.12	472.9 ^b
K-32	24.4 ^c	34.3 ^c	60.7 ^a	5.0 ^b	21.7 ^c	12.6	1.7 ^{ab}	5.2	10.42	1.44	285.8 ^d
K-17	49.2 ^a	62.6 ^a	35.2 ^b	2.2 ^c	51.6 ^a	11.0	1.9 ^a	5.4	9.80	1.33	530.6 ^a
K-39	34.3 ^b	46.6 ^b	24.4 ^c	29.0 ^a	38.2 ^b	8.4	1.8 ^a	5.0	9.38	1.22	388.0 ^c
F test	**	**	**	**	**	ns	*	ns	ns	ns	**
NaCl concentration effects mM NaCl (B)											
0	86.1 ^a	88.6 ^a	3.6 ^a	7.8	78.1 ^a	10.5	2.4 ^a	7.6 ^a	16.27 ^a	1.71 ^a	888.6 ^a
50	60.0 ^b	69.8 ^b	21.0 ^b	9.2	58.8 ^b	11.0	2.3 ^{ab}	6.9 ^b	15.36 ^a	1.59 ^a	638.7 ^b
100	36.5 ^c	52.0 ^c	38.8 ^c	9.2	40.8 ^c	11.2	2.0 ^b	5.4 ^c	9.80 ^b	1.34 ^{ab}	382.3 ^c
150	14.2 ^d	30.8 ^d	60.0 ^d	9.2	21.0 ^d	9.8	1.3 ^c	3.4 ^d	5.61 ^c	1.12 ^b	136.1 ^d
200	5.0 ^e	17.8 ^e	72.2 ^e	10.0	6.2 ^e	11.5	0.7 ^d	2.1 ^e	1.39 ^d	0.61 ^c	50.7 ^e
F test	**	**	**	ns	**	ns	**	**	**	**	**
A × B	**	**	**	ns	**	ns	ns	ns	ns	ns	**

Means followed by the same letter within a row are not significantly different by Duncan's Multiple Range Test at the 5% level; ** - significant at 1% level of probability, * - significant at 5% level of probability and ns - not significant

Tavakkoli et al. (2010) point out that the high concentrations of NaCl reduce seedling growth due to increased concentration of Na⁺ and Cl⁻. For this reason, the seedling growth is reduced (length of root and shoot and fresh and dry weight of root and shoot). Red clover seedlings at the medium with high salt content have lower accumulation of dry matter due to which the seedlings are short and small weight.

Effect of NaCl-induced osmotic stress on seed germination and seedling growth of field pea

Field pea genotypes differ in salinity tolerance at seedling stage. They can be classified into three groups: sensitive, intermediate and resistant to salinity. Previous research shows that Serbian cultivars of field pea are tolerant to salt stress during germination and early embryo growth (Jovičić *et al.*, 2010; Petrović *et al.* 2016). These authors conclude that the germination and seedling quantitative parameters of field pea significantly decrease with increasing NaCl in the medium. The seedling grows slowly under high levels of salinity due to a slow water uptake by seeds, ions are involved in the physiological processes and damage the cell. Thus, Mer *et al.* (2000) report that the Na^+ in the large amounts negatively affects cell division, metabolism and imbalance of other nutrients. Mandić *et al.* (2016) have found genetic variability between field pea cultivars Kosmaj and Letin for germination, seedling parameters, seedling vigor index, relative seedling water content and phytotoxicity of root (Table 4 and 5). According to their results, cv. Kosmaj has significantly higher germination energy, shoot length, shoot fresh and dry weight, relative seedling water content and phytotoxicity of root, while lower germination, root length, root fresh and dry weight and seedling vigor index than cv. Letin. Also, they have found that the all investigated parameters, except phytotoxicity of root and shoot, significantly decrease with increasing osmotic stress. Phytotoxicity of root and shoot significantly increase with increasing osmotic stress.

Table 4. The effects of cultivar and osmotic stress on germination energy (GE), germination (G), root length (RL), shoot length (ShL), root fresh weight (RFW), shoot fresh weight (ShFW), root dry weight (RDW) and shoot dry weight (ShDW).

Factor	GE %	G %	RL cm	ShL cm	RFW mg	ShFW mg	RDW mg	ShDW mg
Cultivar (A)								
Kosmaj	55.4 ^a	92.1 ^b	5.6 ^b	5.6 ^a	49.6 ^b	79.3 ^a	6.3 ^b	7.9 ^a
Letin	27.8 ^b	98.6 ^a	10.3 ^a	2.2 ^b	90.2 ^a	57.5 ^b	13.2 ^a	6.7 ^b
F test	**	**	**	**	**	**	**	*
Osmotic stress, MPa (B)								
0	89.2 ^a	98.9 ^a	11.2 ^a	9.0 ^a	100.2 ^a	136.9 ^a	13.6 ^a	13.6 ^a
-0.3	70.4 ^a	98.1 ^a	9.6 ^b	5.4 ^b	93.1 ^a	112.9 ^b	13.2 ^a	11.4 ^b
-0.6	45.6 ^b	98.0 ^a	9.2 ^b	3.6 ^c	75.7 ^b	71.3 ^c	10.8 ^b	8.1 ^c
-0.9	37.5 ^b	96.8 ^a	7.4 ^c	3.3 ^c	63.6 ^c	54.6 ^c	7.2 ^c	6.0 ^d
-1.2	5.6 ^c	92.9 ^b	5.3 ^d	1.5 ^d	44.6 ^d	23.8 ^d	7.0 ^c	3.1 ^e
-1.5	1.2 ^c	87.5 ^c	5.0 ^d	0.7 ^d	42.1 ^d	10.9 ^d	6.8 ^c	1.6 ^e
F test	**	**	**	**	**	**	**	**
A × B	**	**	**	**	*	ns	**	**

Means followed by the same letter within a row are not significantly different by Duncan's Multiple Range Test at the 5% level; ** - significant at 1% level of probability, * - significant at 5% level of probability and ns - not significant

Table 5. The effects of cultivar and osmotic stress on seedling vigor index (SVI), relative seedling water content (RSWC), phytotoxicity of root (PhR), phytotoxicity of shoot (PhSh) and dry matter stress tolerance index (DMSI) of field pea

Factor	SVI	RSWC %	PhR %	PhSh %	DMSI %
	Cultivar (A)				
Kosmaj	1068.1 ^b	87.8 ^a	44.6 ^a	58.0	61.7
Letin	238.0 ^a	86.1 ^b	15.3 ^b	51.7	63.8
F test	**	**	**	ns	ns
	Osmotic stress, MPa (B)				
0	1991.8 ^a	88.6 ^a	0 ^a	0 ^a	100.0 ^a
-0.3	1474.8 ^b	88.0 ^a	15.7 ^b	29.8 ^b	90.4 ^b
-0.6	1256.4 ^c	87.2 ^a	18.2 ^c	55.5 ^c	68.9 ^c
-0.9	1036.1 ^d	88.8 ^a	34.7 ^c	66.3 ^c	50.4 ^d
-1.2	637.5 ^e	85.4 ^b	54.1 ^d	85.6 ^d	37.1 ^e
-1.5	521.6 ^e	83.7 ^b	57.0 ^d	91.9 ^d	30.0 ^e
F test	**	**	**	**	**
A × B	**	*	**	*	**

Means followed by the same letter within a row are not significantly different by Duncan's Multiple Range Test at the 5% level; ** - significant at 1% level of probability, * - significant at 5% level of probability and ns - not significant

Conclusions

Salt stress causes huge losses in crop production worldwide. The high salt concentration in the solutions had effect on germination and seedling parameters in the crops studied. Significant differences were found in germination of seeds and seedling parameters between genotypes of crops. The choice of crops and genotypes can be a way for their cultivation on salinity soil environments in a sustainable and productive way. It is therefore necessary to start with identification and selection of varieties for cultivation on saline soils during the seedling stage.

Uticaj sonog stresa na klijanje i rast klijanaca nekih useva

Violeta Mandić, Zorica Bijelić, Vesna Krnjaja, Maja Petričević, Aleksandar Stanojković, Marija Gogić, Aleksandar Simić

Rezime

Zaslanjivanje zemljišta je jedan od najvažnijih faktora koji ograničava produktivnost useva. Poznato je da površina poljoprivrednog zemljišta sa visokom

koncentracijom soli raste iz dana u dan. Iz tog razloga, neophodno je pratiti toleranciju useva i genotipova na stres soli jer imaju različitu graničnu osetljivost. Generalno, viši nivoi saliniteta u medijuma negativno utiču na klijavost semena i rast klijanaca većine useva. Klijavost, brzina klijanja i rast klijanaca značajno se smanjuju sa povećanjem koncentracije soli u medijumu. U suštini, salinitet i kiselost negativno utiču na klijanje, što dovodi do lošeg (proređenog) sklopa. Stoga bi razvoj genotipova tolerantnih na soli sa obećavajućim prinosima bilo idealno rešenje za gajenje biljaka na takvim zemljištima, kao i za održivu proizvodnju hrane.

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ORALLY PRESENTED PAPERS

PERSPECTIVES IN PIG FARMING IN GERMANY

Martin Wähner

Anhalt University of Applied Sciences, Bernburg, Germany

Corresponding author: Martin Wähner, martin.waehner@hs-anhalt.de

Review paper

Abstract: Pig production is very intensive and represents an important agricultural activity in Germany. However, changes are needed to improve the economic, social, health and legal problems of pig production. The paper provides an analysis of the problems and proposes changes that should be made to the progress of pig production.

Key words: meat production, pig farming

Introduction

The development of global meat consumption is estimated at over 455 million tones (FAO) by 2050, of which more than 30% for pork and more than 40% for poultry meat. This prognosis results from the analysis of the dramatic development in the present time. The last years have been of lasting effect for German pig farming. After significant growth was recorded from the middle of 1990s to 2013, this development is no longer evident in subsequent years. Since 2017, even a noticeable decline can be observed. It is to be feared that this process will continue. The analysis of social, political and legal situations is necessary to make consequences of possible trends for the next few years. Various reasons have to be mentioned. One of them is that the social demands on livestock husbandry have changed and that these claims as well as the legal framework for the pig owner are completely unclear. This uncertainty implies that fewer investments are made because it is not certain that the right construction will be invested for the next 10 years.

Structural changes in German pig farming

Since 20 years drastic structural changes have occurred in German pig farming.

1. The number of fattening pigs has increased from around 15 million to almost 19 million. The number of pig farmers (holdings) has dropped from 100.000 to less than 20.000 over the same period. Nevertheless, 30% more

carcass weight is produced today than it was 20 years ago. The degree of self-supply with pork has risen from 85% to more than 120%.

2. The development in sow husbandry and piglet production is the opposite. The number of sows declined from nearly 2.7 million to only 1.8 million. The number of sow holders fell from almost 64.000 to less than 8.000. As a result, the average number of sows per farm increased from previously 30 to 220 sows. There is still a drastic difference between East and West Germany. The strong positive development in the fertility of the sows meant that despite the reduced by 30% sowing stock about 3% more piglets are produce.
3. This trend continues. Sows are less, fattening also, but to a much lesser extent. In Germany the import of piglets for finishing has meanwhile increased to over 12 million per year. The proportion of fattening pigs thus increases dramatically in relation to the sows. These structural changes in pig farming were and are accompanied by either particularly positive or particularly negative economic conditions.
4. In the pig fattening production performance increased by more than 20%. Thus, direct costs improved by 15% per animal place. In the working economy a higher efficiency was realized. The result is a cost reduction. The process of growth to bigger farms led to higher profits.
5. In sow husbandry this positive trend did not occur. Here the fixed costs are higher than in pig fattening. Consequently, the profit developed only little even with stock expansion. Piglet production is more costly than pig fattening. Here the relation of money by credits is much higher than in fattening.

In summary, it should be noted that in piglet production, despite the increase of biological performances, the economic situation compared to fattening farms is much less favorable.

For German pig farmers, generally changing social demands become effective. According to *Spandau (2019)*, it concerns the following three problem areas:

- More animal welfare: implementation of measures to increase animal welfare. Moving away from conventional husbandry and stable systems.
- More climate protection: Drastic reduction of emissions (NH_3) from animal husbandry until 2030.

- Changing consumer habits: decrease in meat consumption (health, ethics, demography)
- Discussion about new or other protein sources sufficient food for 10 billion people on earth.
- Legislators are gradually introducing these amended claims into applicable law. Pig farmers need to adapt to this. This concerns the following legislations:
- Construction measures (environmental impact assessment of new construction is sharper)
- Expansion of cumulative effects of animal husbandry in the context of environmentally relevant evaluations
- New evaluation criteria and compulsion to emission reduction measures (exhaust air purification) in barns
- Changes in the Animal Welfare Act and in the Animal Welfare Ordinance with higher habitat requirements (area per animal, climate, roughage), prohibition of tail docking in piglets, piglet castration only under anesthesia, using of antibiotic.

As a consequence, rising production costs for pork arise. But the pork price has been stagnating for 30 years. The change in production structures will become even more effective, especially in piglet production. Above-average production performances are necessary. However, the social demands and the legal framework in livestock husbandry lead to a certain stagnation in the entire production stage. The sow husbandry is increasingly affected by farm closures.

Progress in pig production

There is a new goal. It means to involve the consumer, the market more and more in the conversion of farm animal husbandry. Better produced products should be better paid. In comparison to other industrial products, it is significant that in pork production, not so much the actual product is advertised, than the process of its production. The process quality is above the product quality. Even today, there are a large number of labels (Table 1). Almost all of them focus on the production process, not on a high-quality product. Additional labels will be added in near future.

In this context, demands for improvements in animal welfare, increased food safety, minimization of antibiotic using and increased environmental protection requirements must be realized (*Spandau, 2019*). The multitude of labels has generally complicated the situation for pork. At present time, it is not clear that the implementation of various initiatives to increase animal welfare, are actually

delivering higher profit for the pig farmer. From the point of view of economists, there is no real prospect for the future (*Bongartz, 2019*).

The increasing global consumption of pork is seen as a growth engine for production over and over again. With more environmentally friendly agriculture in Germany and Europe, production for the world market will increasingly take place in other parts of the world where the costs for production are lower than in Europe and Germany.

Table 1. Labels in German pork production (*Spandau, 2019*)

I	II	III	IV
conventional	Improved housing	Outdoor climat & straw	Organic farming
			
Average average production costs per kg carcass weight			
1,50€	1,80€	2,50€	3,50€
Average consumer price per kg of pork tenderloin			
9,- to 14,-€	???	20,- to 25,-€	35,- to 40,-€

In front of this background, measures are necessary which have a cost-limiting but performance-enhancing effect on the national and international competitive situation of companies and pig industry. This situation includes breeding in the first place. With scientific methods today is bred to comprehensive breeding goals. In addition to the economic demands, social demands are taken into account (*Götz et al., 2018*). The success of breeding progress is directly dependent on the accuracy of the breeding evaluation. This can only happen through an accurate and secure performance evaluation. The evaluation should be carried out as directly as possible on the selection candidate itself and at best before the first

selection (*Brandt, 2019*). Functional features are increasingly moving into the center of future-oriented performance evaluation. These include features such as health, fertility, behavior and feed efficiency. However, the requirement mentioned by *Brandt (2019)* is hardly or not realizable for these features.

In order to achieve a good selection intensity, the performance evaluation should be performed on as many animals as possible. Very well but costly is known to be evaluation in test-station. It should be reserved for the breeding animals. The field test is recommended for production animals. In the companies selected for this purpose, the highest requirement is that the data is collected reliably. In addition, the demands on the feature complexes change.

New features such as enjoyment of meat or water retention capacity are important. In terms of fertility, the number of piglets per litter is less interesting today, but rather the ability of a sow to rear 90% of its own born piglets without help (*Hallfarth, 2019*). Other important features are boar taint, tail biting and behavioral traits (for example motherhood). The behavior of pigs is especially important in activities such as weighing, transport or medical treatments. Breeding improvements in behavioral characteristics could shorten routine work and help farmers to be more secure. Also in the group housing of pigs quiet animals contribute considerably to the animal welfare due to a higher stress tolerance. According to *Lindhorst et al. (2019)*, there are low to moderate heritabilities for the behavior of sows when dealing with humans, which allow for selection and lead to no or negligible negative impact on performance characteristics.

Furthermore, with regard to animal health and thus animal welfare, the importance of immunocompetence and immune response in animals is increasing. Thus, genetic analyzes of immune characteristics show a moderate heredity, which is interesting in terms of breeding. Other studies on new traits have shown that the heritabilities for the relatively recent recorded characteristics of stillborn piglets and slightly born piglets are similar to those of the normal traits of live born piglets and raised piglets. A breeding treatment is therefore possible.

For the future, approaches to increase resistance and tolerance with the goal of increasing resilience are important (*Grosse and Müller, 2018; Wähner, 2018; Knap, 2019*). Resilience means robustness or the ability to cushion stressful situations. It can also be described as "performance under practical conditions". Resistance and tolerance fall below: Resistance because it prevents the amount of excitement in the body and tolerance because it makes the body less sensitive to certain levels of pathogens. Here, new breeding techniques could be used effectively. The first PRRS-resistant pig is proof of this (*Knap, 2018*).

The routine use of information from slaughtering to improve animal health is an underutilized field for breeders and pig producers. The data are available in very large numbers, but require extensive statistical workup. Here a closer

cooperation between the slaughter companies and the agriculture is necessary. Important information for breeding, consulting and management can be obtained from this.

In the future, increasing nutrient efficiency and sustainability will be of great economic importance. This requires new breeding strategies to improve feed efficiency. Good conditions have already been created in the past for the sustainable production of products of animal origin. The use of genetic potential, however, is a challenge if you want to feed appropriately and according to the needs of the animal. Technical adjustments in feeding additionally offer important potential for improving nutrient efficiency (*Visscher, 2019*).

More and more important can be following problem. The pig was not originally domesticated as a food competitor to humans, as it is today, but rather to the use of food leftovers and of products or substances that are not useful for humans. In view of the expected global population development and the available resources, it is justified to see the pig also more as a recycler of such residual products.

Interesting interdisciplinary research on the microbiome in pigs reveals new breeding approaches to improving feed efficiency (*Weishaar and Bennewitz, 2019*). Accordingly, intestinal microbes produce messenger substances that affect the entire body of the animal. They also affect the brain. It is spoken of a microbiome-intestinal brain axis. The brain, in turn, acts via the hypothalamic-pituitary-adrenal axis on the composition of the intestinal microbiome. Stressors in pigs lead to the increase of pathogenic microbes in the intestine (*Röhe, 2019*). This in turn can have negative effects on the immune system of the animal as well as on the brain.

Due to the interaction of the brain with the microbiome, it can be assumed that stress also influences the composition of the microbiome and is therefore relevant for animal health. That stress lowers the immune system in animals is well known. For breeding, the intestinal microbiome can be used as a biomarker to improve resistance to pathogenic intestinal bacteria and as an indirect biomarker for increased tolerance to stress (*Röhe, 2019*).

Animal welfare and animal welfare are very important for the future development of the pig industry in Germany and Central Europe. Various projects, which have been promoted by the government and by private companies, will deal with sub-topics on these large complexes. Various farrowing systems are being examined with regard to production indicators, animal behavior, animal losses, animal health, economic and safety issues for people. Despite the acceptance of alternative husbandry methods for suckling sows, such as movement farrowing box or free farrowing system, suckling piglet losses due to crush, which are above the values of conventional husbandry, cannot be mediated. With regard to animal

welfare, the movement farrowing box (picture 1) represents a compromise between piglet protection cage and free farrowing system. However, the investment costs will be significantly higher than those for a box with a piglet protection cage (Burfeind, 2019; Meyer, 2019).



Figure 1. Movement farrowing box

Objective data that is routinely incurred is a great reservoir for information on a lot of problems. Animal welfare is one of them. The automatic detection of animal welfare indicators at the slaughterhouse can be a perfect measure for this. Compared to staff assessment, auto-detection ensures consistent and comparable results. Good experiences are available from some poultry slaughterhouses. In pig slaughter, the camera-based system has been successfully established for the diagnosis of ear necrosis and tail necrosis. In the future, it may be possible to use this technical system to detect other indicators, for example joint changes (Kemper, 2019).

Conclusion

Farm animal husbandry, especially pig farming, is the focus of public discussion. Massive challenges have to be registered in view of the global population development and the changed social demands. At present time, there is a major change in production structures. Above-average production performance and significant structural changes are necessary. Farm animals, including pigs, have been domesticated and bred for using by humans and for human consumption. From the point of view of animal and environmental protection it must be possible to control food competition with humans and environmental pollution farm animals.

In present time breeding goals have become more comprehensive character than in previous years. In addition to the economic conditions, social demands are taken into account. On the one hand, further developments will take place in the genetic field, where in the future epigenetics, gene editing and the genetics of the microbiome will be researched and used. Equally important will be the improved possibilities of capturing features through digitization and routine technical procedures for breeding, consulting and management.

Perspektive u svinjarstvu u Nemačkoj

Martin Wähner

Rezime

Proizvodnja svinja je veoma intenzivna i predstavlja veoma važnu delatnost poljoprivrede u Nemačkoj. Međutim, neophodne su promene koje bi poboljšale rešavanje ekonomskih, socijalnih, zdravstvenih i pravnih problema u proizvodnji svinja. U radu je data analiza problema i predlog promena koje bi trebalo ostvariti za napredak proizvodnje u svinjarstvu.

Ključne reči: proizvodnja mesa, svinjarstvo

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BODY SCORE CONDITION OF SOWS AND THE THIN SOW SYNDROME AS HEALTH PROBLEMS ON COMMERCIAL FARMS

Jovan Bojkovski¹, Jasna Prodanov-Radulović², Milica Živkov-Baloš², Radiša Prodanović¹, Sreten Nedić¹, Sveta Arsić¹, Ivan Vujanac¹, Ivan Dobrosavljević³, Suzana Đedović⁴, Renata Relić⁵, Dušica Ostojić Andrić⁶

¹University of Belgrade, Faculty of Veterinary Medicine, Bulevar oslobođenja 18, Belgrade, Serbia

²Scientific Veterinary Institute "Novi Sad" Rumenički put, 20 Novi Sad, Serbia

³Specialistic Veterinary institute, Dunavska, 89 Požarevac, Serbia

⁴Institute for Pesticide and Environmental Protection · Laboratory for Applied Zoology, Banatska 316, Belgrade- Zemun, Serbia

⁵University of Belgrade, Faculty of Agriculture, Nemanjina, 6, Belgrade- Zemun, Serbia

⁶Institute for Animal Husbandry, Autoput 16, Belgrade-Zemun, Serbia

Corresponding author: Jovan Bojkovski, bojkovski@vet.bg.ac.rs

Review paper

Abstract: In this article we presented body score condition of sows and thin sow syndrome. In modern pig farming more and more attention is paid to body condition score. On most commercial farms feeding of gilts and sows is based on body condition. The goal is that sow does not gain or lose too much of a body weight between farrowing and insemination. Maintenance of body weight (condition) of sows within the optimum value (3 points in the time of farrowing and 2.5 during mating) over their lifetime can result in optimal reproductive results. In contrast, inadequate control of condition of the sow may lead to difficulty in farrowing and occurrence of health problems. The syndrome of thin sows is a disease of complex etiology. In 75% of cases, the main causes are qualitative and quantitative malnutrition, also unfavorable housing conditions for sows. Less frequently, the syndrome can be caused by presence of endoparasite *Hyostrogylus rubidus*. In the presenting article during a year period, the occurrence of weight loss in sows during lactation at one commercial farm was observed. The health status of lactating sows and piglets were also registered. The loss of body weight was mostly detected in sows in the second lactation. Corrections in the feeding technology and better conditions for sows during lactation period are crucial for managing the thin sow problem on the commercial farm.

Key words: body score condition, thin sow syndrome, sows

Introduction

Today more attention is paid to assessment of body condition score of pigs. Body condition score is the most reliably performed by measurement of the thickness of adipose tissue in the back of sows using ultrasound machines, but nevertheless it is often done only visually (*Hutu and Onan, 2008; Bojkovski et al., 2003, 2013; Petrujkić et al., 2011; Maes et al., 2004*). Visual grading system of body condition can be subjective and in large percentage measures may depend on the competence of assessors. For example, in one Canadian study, sows with body condition score of 3 had adipose tissue in the back from 9 to 28 mm. Additional information on the measurement of body fat on back and body condition of sows on three farms in the state Minnesota (USA) indicate that between 18 and 40 percent of sows have back fat smaller than 13 mm. Also, it is measured that sows with body condition score of 3 have from 9 to 24 mm of adipose tissue on the back. American and Canadian authors recommend that less than 20% of sows on the farm should have less than 15 mm of adipose tissue in the back. Based on this data it is tendency to develop nutritional method in sows during pregnancy which is going to be based on the thickness of adipose tissue on the back (i.e. body condition score). The goal is to minimize the variations in quantity of adipose tissue on the back between pregnancy sows so that at farrowing stage it is approximately 19 mm (at last rib). American researchers measured adipose tissue on the back and estimated body condition in total of 731 sows with goal to determinate „accuracy of nutrition” of pregnant sows based on the body condition score. The body condition score was in weak correlation ($r^2 = 0.19$) with thickness of adipose tissue on the back. For example, sows with body condition score of 3 had adipose tissue thickness between 7.5 and 23 mm. This proves that it is necessary to find more objective procedure for measurement of body condition (as ultrasound for example) in order to adjust nutrition level and minimize variation in thickness of adipose tissue of sows (*Coffey and Parker 1999; Petrujkić et al., 2011; Straw, 2006; Simeunović et al., 2016*).

The thin sow syndrome is a condition characterized by anorexia and weight loss. Usually, it appears as a consequence of errors in sows' nutrition during partition and lactating period, as well as different failures in production technology on the farm. The condition may results as a combination of parasitism (helminth or mange), low environmental temperatures and inadequate feed intake, particularly during lactation. I mean real Serbia climatic conditions, a more frequent occurrence of this syndrome is observed during the winter months as a result of inadequate environmental conditions (temperature less than 21 °C and inappropriate ventilation level) in pig barns. The syndrome may also occur during or after

recovery period from some infective diseases, such as influenza (Lipej, 2015). Some authors suggested that some parasitic infections may also play role in pathogenesis of the thin sow syndrome (Šamanc, 2009; Lipej, 2015). From the group of endoparasites, the most frequent causes are gastric parasites *Hyostrogylus rubidus*, and from the group of ectoparasites - causative agents may be itch mites (Šamanc, 2009). However, the parasitism is less important when adequate prophylactic measures and therapy are routinely carried out in the commercial swine production. The thin sow syndrome is most often clinically observed after first and second farrowing or lactation period. This phenomenon is one of the main reasons for the exclusion of a large number of sows from reproduction, after first and second farrowing. Clinical signs of suboptimal condition include increased weaning to service intervals, small litters and low weaning weights. Piglets of sows in suboptimal condition may be restless and demand milk more frequently (Petrujkić et al., 2011). During lactation, the nutritional needs are high, and inappropriate diet is one of the most frequent reasons of significant decrease of sow body condition. If this phenomenon lasts longer, and if the deficit of nutrients is more pronounced, a "thin sow syndrome" occurs. Thin sows may be identified by observation and systematic condition scoring of the herd. Pressure sores in sows at weaning also indicate poor body condition. Some production parameters can also be used to detect thin sows. The particular susceptibility of young gilts is due, among other things, their unequivocal use of large quantities of food during first lactation and small body reserves, which should be considered that they body is still developing. In extreme cases, the weight loss can involve 30- 90% of sows in one herd (Šamanc, 2009; Lipej, 2015; Bojkovski et al., 2015, 2016a,b.). The "thin sow syndrome" is a significant welfare problem and some data can be found in paper by Relić et al. (2016). In this paper, situation concerning body and health condition of lactating sows at one commercial Serbian farm is discussed.

Material and Methods

In our research we estimated body condition score of sows at one commercial type farm. Assessment of body condition score is performed visually in 47 sows at 90th day of gestation. Statistical data are processed using IBM SPSS Statistics 20 and Microsoft Excel 2003 program and as method we used ANOVA to determine statistical differences between parities.

The material for this research included animals from one commercial swine farm (capacity 500 sows), where health disorders i.e. clinical and gross pathology signs resembling to the problem of thin sow syndrome and/or sudden body loss in large number farrowed females were detected. Research methods included

epidemiological (farm production data analysis) and clinical evaluation, and gross pathological examination (i.e. post mortem lesions detection in case of sow death). In the pig units, the following details were ascertained by the interview and from the farm records: number and category of pigs in the unit, production details (breeding, finishing unit, nucleus or commercial), disease status, current veterinary health plan (vaccination programs, routine medication), biosecurity protocols and feeding system used. Furthermore, data on air temperature and ventilation, stocking density, type of bedding and hygiene level in the unit were collected. The animals were observed and inspected for clinical signs of disease and abnormal behavior.

Results and Discussion

It is not easy to evaluate the body condition in an objective way under practical circumstances. In many herds, body condition is evaluated by the pig producer by visual scoring, on a scale ranging from 1 to 5. Although visual scoring systems may work well in some herds, e.g. in outdoor systems, they have several disadvantages. First, a sow that appears to be thin can still have a fairly high amount of back fat (*Muirhead and Alexander, 1977*). Second, it is a subjective and inaccurate method that largely depends upon the scoring skills of the person. Finally, when visual scoring is performed by the pig producer in the same herd over time, it is likely that less attention will be paid to deviations from the optimal condition due to herd blindness. Determining the optimal body condition by visual scoring is particularly difficult in herds with sows of less than one type of breed because of the inherent variation in conformation existing between breeds (*Whittemore and Schofield, 2000*). In the experiment we had total of 47 sows in which parity ranged between 2 and 6. Applying ANOVA method there were no significant differences between parities. The number of live born piglets ranged from 9 to 22 piglets and the number of dead born piglets ranged from 0 to 6. It was found that the highest percentage of piglets born alive existed in sows of parity 5 as shown in figure 1. Body condition score at the 90th day of gestation was 5 in 7 sows, estimated body condition 4 had 16 sows and 24 sows had body condition score 3. In our experiment, we found that 7 sows had a body score condition 5. Sows farrowing to go with body score condition 5 have health- reproduction disorders. For this reason, we have to try to not go to the farrowing sows with the assessment of body score condition 5. With a score of body condition 3 were 24 sows. Body score condition 3 is optimal. In our survey 16 sows were a body score condition 4. Body score condition 4 gives a chance for the correction of the meal to farrowing, sows that suffer of health-reproduction problems.

Data from the farm records and current situation on the farm indicated that zoohygenic, prophylactic and biosecurity measures were not carried out in an

adequate regime. Also, the regime of preparing the sows for farrowing and the feeding is not adequately regulated. Decreases of feed intake and weight loss in the lactation period were noticed in animals after the 1st, 3rd, 4th and 5th farrowing. In the cases when gilt condition and nutrition in the lactation period was inadequate, the second litter was smaller than the first. Certainly, that low number of newborn may reflect overall sow condition at service. The extended weaning to service intervals and low weaning weights were connected to the poor body condition. The litters of the lean sows were smaller, and in the case of the pig's rejection, it attained less body weight. In some cases, problems with conception or early abortion in pregnant sows were also noticed. On the sows' body, some skin changes (wrinkles and different types of lesions) and swellings were noticed, as well as clogged and dirty hair. The lesions were formed dominantly at the point of bone compression. Most often, thin sows were found in a position lying on the sternum. In some cases, it was very difficult for these animals to take a standing position in the box i.e. The detected gross pathology post mortal lesions were grossly classified as poor body condition, low fat thickness and as an increased incidence and extent of skin lesions, especially over shoulders and hips where pressure sores can develop. Anorexia (loss of appetite) in sows develops after farrowing as part of a "thin sow syndrome", and as a result there is an intense loss of body weight. The clinical signs of this syndrome, observed in the examined cases, show an unusual similarity to the clinical signs of anorexic nerve (Anorexia Nervosa). In addition to losing appetite and body weight, sows limit the intake of normal foods and consume large quantities of straw. Animals spend more time on non-intrusive hyperactive behavior, constantly moving inside the box. The sows affected by anorexic nervousness are easily recognized by the prominent backbone of the spine and their rough and long hair (*Treasure and Owen, 1995*), which is also in line with the observed changes in our survey experiment.

Many factors may affect the appetite of sows in lactation, and the most important are: consumption of the food during pregnancy, air temperature and ventilation in the pig barn, energy level in the meal and the number of feeding per day (*Kovčín, 1993; Bojkovski et al., 2018*). The most powerful effect on the level of consumption has the level of energy in the meal, so if lactating sows are not allowed to eat *ad libitum* or close to it, than production of milk, body weight and level of body reserves decreases. On the other hand, the needs in nutrients during lactation vary and depend on the concentration of energy in the meal and the previous feeding of the sow. In practical nutrition, the highest efficiency of energy consumption from meals is achieved by controlled diet during gravidity in order to minimize the mobilization of body depots of fat during lactation (*Jovanović et al., 2001*). Cases of severe constipation can be avoided by increasing the amount of dietary fiber during the last phase of suppression (*Treasure and Owen, 1997*;

Young et al., 2001; Tabeling et al., 2003; Kokkonen et al., 2009). Ensuring optimum levels of dietary fiber improves the functioning of the bowel and may significantly reduce the degree of constipation. It seems that the use of high-fiber meals in the form of coarse humpy 128 nutrients is a useful strategy for improving the health of pigs (*Peltoniemi et al., 2016*).

Conclusion

According to our results sows with parity 5 gave the best results. Our recommendation for commercial farms is to introduce body condition score in daily routine.

“Thin sow syndrome” on commercial farms can be prevented by correction in the feeding technology and feeding sows during the lactation period. It is recommended to carry out energy and protein balanced diet during gravidity and lactation, and restrictive diet, the first few days after partitition. In the critical period, at the beginning of lactation, the health control of sows should be performed regularly on a daily basis in order to spot and detect the earliest symptoms of the disease (long sleeping periods, reduced appetite and constipation).

Certainly that improved sow nutrition at key stages in the breeding cycle will help improve the number, birth weights and piglet vitality. This breakthrough in sow nutrition can help the sow in supporting larger litters, from birth to weaning.

Ocena telesne kondicije krmača i sindrom mršavih krmača kao zdravstveni problemi na komercijalnim farmama

Jovan Bojkovski, Jasna Prodanov-Radulović, Milica Živkov-Baloš, Radiša Prodanović, Sreten Nedić, Sveta Arsić, Ivan Vujanac, Ivan Dobrosavljević, Suzana Dedović, Renata Relić, Dušica Ostojić-Andrić

Rezime

U ovom članku predstavili smo ocenu telesne kondicije kod krmača i sindroma mršavih krmača. U savremenom svinjarstvu sve se više pažnje posvećuje oceni telesne kondicije krmača. Na većini komercijalnih farmi hranjenje nazimica i krmača zasniva se na oceni telesne kondicije. Cilj je da krmača ne dobije ili izgubi previše telesne mase između osemenjivanja i prašenja. Održavanje telesne mase krmača unutar optimalne vrednosti (3 boda tokom pranja i 2.5 tokom

osemenjavanja) tokom njihovog životnog veka mogu da posluže optimalnim reproduktivnim rezultatima. Suprotno tome, neadekvatna kontrola stanja krmače može dovesti do pojave zdravstvenih problema. Sindrom mršavih krmača je bolest složene etiologije. U 75% slučajeva glavni uzroci su kvalitativna i kvantitativna pothranjenost, takođe nepovoljni uslovi za krmače. Sindrom rede može biti uzrokovan prisustvom endoparazita *Hiostrongilus rubidus*. U toku jedne kalendarske godine praćena je pojava gubitka telesne mase kod krmača tokom dojenja na jednoj komercijalnoj farmi. Takođe je registrovano i zdravstveno stanje krmača u laktaciji i prasadi na sasi. Gubitak telesne mase uglavnom je otkriven kod krmača u drugoj laktaciji. Korekcija u tehnologiji ishrane i bolji smeštajni uslovi za krmače tokom perioda dojenja ključni su u rešavanju problema sindroma mršavih krmača.

Ključne reči: ocena telesne kondicije, sindrom mršavih krmača, krmače

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A COMBINATION OF KARYOTYPING AND MOLECULAR METHODS COULD INCREASE THE DETECTION ACCURACY OF CHROMOSOMAL ABNORMALITIES IN HORSES: A CASE REPORT IN PURA RAZA ESPAÑOL HORSE

Miguel Moreno-Millán¹, Delia Saleno¹, Gabriel Anaya¹, Yamila Pirosanto², Florencia Azcona², Olivia Marcuzzi², Antonio Molina¹, Sebastián Demyda-Peyrás^{1,2}

¹ Department of Genetics, University of Córdoba, Córdoba, Spain

² Department of Animal Production Faculty of Veterinary Sciences, National University of La Plata, La Plata, Buenos Aires, Argentina

Corresponding author: Sebastián Demyda Peyrás, sdemyda@fcv.unlp.edu.ar

Original scientific paper

Abstract: Chromosomal abnormalities associated with the sex-pair are a major cause of infertility in equines. However, its complex karyotype made that the percentage of individuals with fertility problems which are chromosomally evaluated is still scarce. To this end, we recently developed a new molecular methodology based on short tandem repeats analysis which allows determining most of these abnormalities in a simple way. But its efficiency sensibility is limited in the case of mosaicisms. In this study, we combined conventional karyotyping and molecular methods, to evaluate the chromosomal complements of a seven years old mare Pura Raza Español which showed a prominent clitoris which naturally protruded by the vulva and absence of estrous. Ultrasonographic findings also showed a small uterus and bilateral ovary hypoplasia. Seven STR markers were evaluated in DNA obtained separately from blood and hair, as well as *ZFX* and *SRY* genes by PCR and capillary electrophoresis. Additionally, Giemsa stained karyotyping was performed in order to determine the chromosomal complements. Molecular results showed a normal mare profile, in both DNA samples, with negative results for *AMYY* and *SRY* and positive results for *AMX*. Similarly, STRmarkers showed bi-allelic or mono-allelic results, which are not compatible with chimerism. However, karyotyping revealed the presence of two cellular lines: one compatible with a normal mare (64,XX) and an additional line compatible with Turner's syndrome (63,X) in a proportion of 70/30 respectively. In this case, the absence of differences between molecular profiles in two different DNA samples ruled out the presence of chimerism, determining the existence of sex-pair

mosaicism (64,XX/63,X) as the cause of the mare infertility. Therefore, we demonstrated that the use of combined cytogenetical and molecular methodologies improve the diagnostic results of chromosomally aberrant horses.

Keywords: chromosomal abnormalities; horses; STR markers, karyotyping, mosaicism.

Introduction

Chromosomal abnormalities have been described as a major cause of infertility in the domestic horse (*Lear and McGee, 2012*). This domestic species is, by far, the livestock animal in which more cases have been reported, most of them being associated to aberrations in the replication of the sex pair, producing different kind of disorders in the sexual development, such as the Turner's syndrome (monosomy of the ECA; 64,X), the Klinefelter syndrome, (65,XXY) or diverse grades of sex reversal individuals and chimerism (*Villagómez et al., 2011*). Their detection is not simple, since horse karyotype is entangled and complex, thus requiring a high grade of expertise to determine accurately the presence of such abnormalities. However, new molecular approaches have been recently developed allowing to determine this kind of abnormalities based on DNA samples, avoiding the long and tedious process of karyotyping (*Anaya et al., 2017*). In this sense, our group has recently analyzed more than 100,000 individuals of the Pura Raza Española horse, detecting 20% of chimerism among twin foals (*Anaya et al., 2018*). Despite these results, the percentage of individuals with fertility problems which are chromosomally evaluated is still scarce. To this end, we recently developed a new molecular methodology based on short tandem repeats analysis which allows determining most of this abnormality in a simple way. But its efficiency sensibility is limited in the case of mosaicisms. Therefore, a combination of classical and molecular tools is still the best option for its detection.

Horses are characterized by moderate fertility in comparison with the rest of the domestic species. Moreover, the prevalence of reproductive problems associated with genetic conditions, and particularly with chromosomal abnormalities, are highly increased. Therefore, the detection of this pathologies could, in an early and effective way could be an interesting tool to the breeders in order to correct manage those animals based on his real possibilities.

Materials and Methods

In this study, we evaluated a 7years old Pura Raza Español mare which showed phenotypical abnormalities in their internal and external reproductive organs. An exhaustive examination was made by rectal palpation and ultrasonography, following our standard procedures in equids (*Dorado et al., 2017*), using an Aloka SSD 500 ultrasound (ALOKA Co. Ltd., Tokyo, Japan). Sexual cycle status was the same determinations and classification of the sexual cycle status were performed, according to *Brinsko et al. (2011)*, in eight contemporary and cycling jennies belonging to the same herd and then statistically compared with our case by confidence interval test. At the examination, blood and hair samples were collected in order to obtain DNA.

Molecular analysis

DNA was obtained from blood and hair follicles separately using the Tissue Genomic DNA Purification Kit and the Blood Genomic DNA Extraction Kit (Canvax Biotech. SL, Cordoba, Spain), respectively, following the manufacturer's instructions. DNA was quantified using a Nanodrop™ 2000 (Thermo Scientific, Madrid, Spain) and stored at -20°C until use.

Six microsatellite markers, five X- linked (*LEX003*, *LEX0026*, *TKY38*, *TKY270*, and *UCEDQ502*) and one Y- linked (*ECAYM2*), were analyzed using our methodology (*Demyda-Peyrás et al., 2014*) using 6- carboxyfluorescein (FAM™, Sigma- Aldrich Biochemie GmbH, Hamburg, Germany) or HEX Phosphoramidite dye (HEX™, Applied Biosystems) labeled primers. PCR determinations were also performed following our standard procedure. Amplified products were genotyped by capillary electrophoresis using an Applied Biosystems 3130 xl DNA sequencer (SCAI genomics core, University of Cordoba, Spain). Allele allocation was performed using genotypes 4.0 software package using a LIZ 500- bp internal size standard (Applied Biosystems, Madrid Spain). Additionally, three different sex-linked PCR products were determined: (*SRY*, ECAY-linked) and two variants of amelogenin *AMX* and *AMY*, which were associated with *ECAX* and *ECAY* respectively. Those genes were amplified according to (*Hasegawa et al., 2000*) using the 21 exon of the *KIT* gene as positive (*Haase et al., 2007*).

Chromosomal analysis

Cytogenetic analysis was performed according to our protocol for horses (*Demyda-Peyrás et al., 2014*) on metaphase spreads obtained by culturing blood lymphocytes. The preparations were obtained by dropping 100 µl of the cell suspension onto wet slides. At least, 100 Giemsa- stained karyotypes were analyzed in a Cytovision™ platform (Leica, Madrid, Spain) and characterized

based on the standard nomenclature (*Richer et al., 1990*). The percentage of numerical abnormalities (metaphases different to $2n = 64$) was recorded.

Results

The examination revealed a prominent clitoris which naturally protruded from the vulva and a complete absence of estrous. Ultrasonographic findings also showed a small uterus and bilateral ovary hypoplasia. Those findings agree with previous reports of infertile individuals in the species associated with chromosomal abnormalities such as Turner's syndrome and SDS horses (*Villagómez et al., 2011*). Chromosomal complements also showed the existence of two different cell lines in the individual: a normal female line (64,XX) and an ECAX monosomic line (63,X) with a prevalence of 67%/33% respectively. However, in this case, molecular analysis showed the profile of a normal mare in all the determinations made (3 by-allelic and two mono-allelic STR markers on ECAX, positive for *AMX* and *KIT* and negative for *SRY* and *AMY* fragments). It was also noteworthy that hair and blood molecular profiles were identical. Based on those results, the individual was diagnosed as a 64,XX/63,X mosaicism.

Discussion

The domestic horse is characterized by an increased prevalence of chromosomal abnormalities. In particular, mosaicism was detected in several breeds and associated with reproductive failures (*Wieczorek et al., 2001; Bugno et al., 2007; Pieńkowska-Schelling et al., 2016*). More recently a Sorraia horse, a critically endangered horse breed of Portugal, in which a case similar to our findings was described (*Kjöllerström et al., 2011*), as well as two related mares with showed the same chromosomal complements observed in our case (*Neuhauser et al., 2019*). But this kind of abnormality was also associated with sterility for the first time in the domestic donkey (*Equus asinus*) just two years ago (*Dorado et al., 2017*). Despite the fact that all previous reports of this syndrome were observed along with infertility in the equids, the degree of mosaicism (the prevalence of the aberrant cell line in comparison with the normal one) showed some effect in the degree of phenotypic alterations in humans (*Sarkar and Marimuthu, 1983*). In fact, it was also described that tissue-specific mosaicism could be observed in 45,X (Turner's) humans (*Nazarenko et al., 1999*). In our study, the prevalence observed was higher than previous reports, which could explain infertility, morphological abnormalities and the absence of sexual cyclicity observed in the mare.

One of the advantages of molecular tools employed to determine chromosomal abnormalities in this species is the low cost and reliability of the results obtained based on DNA techniques (Anaya *et al.*, 2017). Despite that the use of SNP-array was employed previously in the horse (Holl *et al.*, 2013), STR-based methods are more efficient and cost-effective in this kind of diagnostics. But their ability to determine differences between mosaicism and chimerism is a plus that could not be underestimated since these two different pathologies cannot be discriminated by normal karyotyping since the origin or the cell lines cannot be determined. As an example, we employed this technique satisfactorily to discriminate the first case of chimerism in which the two cell lines, one normal and one aberrant, were originated in two different horse embryos (chimerism), only by comparing the results obtained in blood and hair DNA. Using the same technique, we detected several cases of chimerism in PRE horses without showing any sign of morphological abnormalities (Anaya *et al.*, 2018), probably because the chimerism was only on the blood tissue. Despite that, there are few reports in which ex chimerism was associated to morphological abnormalities in horses (Dunn *et al.*, 1981; Albarella *et al.*, 2018), but only in the last case, the existence of true chimerism was assessed by comparing samples of two different tissues. On the contrary, mosaicism cannot be detected by molecular method since the different cell lines individual were originated by an abnormal division of a single individual, and therefore, all the molecular markers will be identical. Therefore, classical cytogenetics techniques are the proper methodology to fetch a preliminary diagnostic in this kind of individuals.

Conclusion

In this study, we demonstrated that the use of combined cytogenetic methodologies, both classical and molecular are necessary to discriminate, with a high degree of certainty, the presence of mosaicism or chimerism in horses. Further, we also added a new case that reaffirms the hypothesis that sex mosaicism is associated with infertility in the domestic horse.

Kombinacija kariotipizacije i molekularnih metoda bi mogla povećati tačnost detekcije hromozomskih nepravilnosti kod konja: prikaz slučaja konja pura raza español

Miguel Moreno-Millán, Delia Saleno, Gabriel Anaya, Yamila Piroso, Florencia Azcona, Olivia Marcuzzi, Antonio Molina, Sebastián Demyda-Peyrás

Rezime

Hromozomske nepravilnosti povezane s rodnim parom glavni su uzrok neplodnosti kod kopitara. Međutim, njegov složen kariotip navodi da je procenat jedinki sa problemima plodnosti koje su hromozomski procenjene i dalje mali. U tom cilju, nedavno smo razvili novu molekularnu metodologiju zasnovanu na analizi kratkih tandem ponavljanja, koja omogućava utvrđivanje većine ovih abnormalnosti na jednostavan način. Ali njegova osetljivost je ograničena u slučaju mozaizama. U ovom istraživanju kombinovali smo konvencionalne kariotipičke i molekularne metode da bismo procenili hromozomske komplemente sedmogodišnje kobile Pura Raza Español koja je pokazala istaknuti klitoris koji je prirodno izbočen vulvom i odsustvom estroze. Ultrazvučni nalaz takođe je pokazao malu hipoplaziju maternice i obostranu hipoplaziju jajnika. Sedam STR markera procenjeno je u DNK dobijenom odvojeno od krvi i dlaka, kao i u *ZFX* i *SRY* genima pomoću PCR i kapilarne elektroforeze. Pored toga, urađeno je kariotipiranje obojeno sa Giemsa kako bi se odredili hromozomski komplementi. Molekularni rezultati pokazali su normalan profil kobile u oba DNK uzorka, sa negativnim rezultatima za *AMYY* i *SRY* i pozitivnim rezultatima za *AMX*. Slično tome, STR markeri su pokazali bi-alelne ili mono-alelne rezultate, koji nisu kompatibilni sa himerizmom. Međutim, kariotipizacija je otkrila prisustvo dve ćelijske linije: jedne kompatibilne sa normalnom kobilom (64, XX) i dodatne linije kompatibilne sa Turnerovim sindrom (63, X) u srazmeri 70/30, respektivno. U ovom slučaju, odsustvo razlika između molekularnih profila u dva različita uzorka DNK isključilo je prisustvo himerizma, određujući postojanje mozaičnosti spolova (64, XX/63, X) kao uzroka neplodnosti kobile. Stoga smo pokazali da upotreba kombinovanih citogenetičkih i molekularnih metodologija poboljšava dijagnostičke rezultate hromozomski aberantnih konja.

Ključne reči: hromozomske nepravilnosti, konji, STR markeri, kariotipiranje, mozaicizam.

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EPIDEMIOLOGICAL AND THERAPEUTICAL STUDIES ON STRONGYLE INFECTION OF DONKEYS IN EGYPT

Maha I. Hamed¹, Taha A. A. El-Allawy¹, Esraa A. Hassnein²

¹Animal Medicine Department (Infectious Diseases)- Faculty of Veterinary Medicine- Assiut University- Assiut- Egypt, 71526.

²Veterinarian in El-Kharga veterinary Administration, El Wadi El Gadid Veterinary Medicine Directorate, Wadi G, Egypt

Corresponding author: Maha I. Hamed, maha.ibrahim@aun.edu.eg

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Abstract: Strongyle species infestation considered the main parasitic problem in equines specially donkeys. A total number of 215 donkeys of different sexes, ages and in different locations at El Kharga city, El Wadi El Gadid Governorate, Egypt were examined for detection of *Strongyle species* infection. Fecal samples were collected and examined using floatation test and egg count was done using McMaster technique. The overall prevalence of *Strongyle species* was 73.02% of examined donkeys. 35.03% of infested donkeys showed mild infection, 20.38% showed moderate infection and 44.59% showed severe infection. Prevalence of *Strongyle species* was 75.56% in female donkeys and 71.2% in males. The highest prevalence rate was recorded at Al-Sherka55 (100%) followed by Al-Thawra areas (84.62%). Animals under 3 years old had a slightly higher prevalence than animals 3 – 8 years old, and donkeys > 8 years old showed no infection rate. The therapeutic efficacy of ivermectin, doramectin and pyrantel were evaluated in a controlled experiment involving 28 donkeys naturally infected with *Strongyle species* worms. Doramectin was highly efficient against *Strongyle species* worm infection than ivermectin and pyrantel. In ivermectin treated group, the mean FECR% reduced on day 28 to be 91.6%. Also, it has been found that small Strongyle EPG counts are returned much quicker than when the drug first was marketed. Mean FECR % in pyrantel treated animals was less than 90% for the whole 28 days of treatment. In conclusion, Strongyle species infection was prevalent in donkeys in El-Wadi El-Gadid governorate, and treatment with ivermectin and pyrantel showed anthelmintic resistance.

Keywords: Strongyles, anthelmintic resistance, donkeys, Egypt, ivermectin

Introduction

Donkeys represent the most valuable and low-cost mean of transportation for man, they are adapted to poor quality grass or forages. Donkeys are easy to manage and spread widely in Africa and rural areas (Ahmed *et al.*, 2011). In Egypt, horses and mules are less in population than donkeys (Mohsen, 1998), the latter is irreplaceable animals as they help the farmers in the field work, packing, riding, carting, and ploughing (Yoseph *et al.*, 2005; Woodford, 2009). The number of equines in El Kharga city of EL Wadi El Gadid governorate is 1468 comprising 79 horses, 6 mules and 1383 donkeys (District unpublished report, 2018).

Parasitic infection is the main problem that affecting equines specially donkeys. It affects animal breeding as it lowers fertility and reduces the animal's ability to work (Ahmed *et al.*, 2011). Donkeys are considered hosts to a large number of parasites include red worms (Strongyles), round worms (*Parascaris equorum*), tape worms (*Anoplocephala species*), pin worms (*Oxyuris equi*), thread worms (*Strongyloides westeri*), lung worms (*Dictyocaulus arnfieldi*) liver flukes (*Fasciola hepatica*) and larvae of bot flies (*Gasterophilus species*) (Eckert *et al.*, 2008). Family Strongylidae is the most common parasite infection of donkeys in the veterinary field (Ali *et al.*, 2001; Eysker *et al.*, 1992). It is usually found in the large intestine of the animal and adults commonly called Strongyle nematodes or Strongyles. Strongyles that infect equines are classified into subfamilies Strongylinae and Cyathostominae or large Strongyles and small Strongyles respectively, and both Strongyles are highly pathogenic parasites (Duncan, 1973; Drudge and Lyons, 1977; Soulsby, 1982; Austin, 1994).

Infection with Strongyle worms causes health problems in equines and its clinical signs are: colic, emaciation, decrease in physical performance, indigestion, abdominal distention, intestinal rupture, and death (Brady and Nichols, 2009). These worms usually suck blood resulting in anemia, destruct the intestinal mucosa resulting in inability to absorb nutrients, obstruct the intestinal lumen and damage other tissues during larvae migration (Brander *et al.*, 1982). The pathogenic effect of Strongyles is dependent on the immunity of the host, number of the ingested larvae species, and nutrition status of the animal (Soulsby, 1982).

Currently there are three broad spectrum drug classes which are the benzimidazoles, the tetrahydropyrimidines (pyrantel) and the macrocyclic lactones available for treatment of parasitic infections in equines (Gasser *et al.*, 2004) and (Fischer, 2013). Management practices and drug availability are important factors in determining the timing and number of treatments the animal should receive (Ali *et al.*, 2015).

A serious problem facing the control of gastrointestinal nematodes infection in donkeys specially Cyathostomines is the anthelmintic resistance. *Lyons et al. (1999)* reported benzimidazoles resistance as well as piperazines and tetrahydropyrimidines. Resistance of small Strongyles (Cyathostomines) to anthelmintics are now known world-wide (*Kaplan, 2002; Kaplan, 2004*). Resistance to benzimidazoles was reported before with five species of Cyathostomines (*Drudge et al., 1979*). Therefore, macrocyclic lactones were used extensively during the past 20 years for treatment of Strongyles infection in equines (*Fischer, 2013*). Ivermectin resistance was first reported in horses in Brazil by *Molento et al. (2006)*. The only reported case of resistance against macrocyclic lactones in donkey was in the United Kingdom (*Trawford et al., 2005*).

There is no available data about Strongyle infection in donkeys at El-Kharga city, El Wadi El Gadid governorate, Egypt. Also, only few reports are available about the efficacy of the anthelmintic drugs used for treatment of infested donkeys in that area. Therefore, the aim of this study was to determine the prevalence of strongyle infection in donkeys and to investigate the efficacy of the anthelmintic drugs used for treatment of infested donkeys in that area.

Materials and Methods

I. Prevalence of Strongyle species infection in donkeys.

1. Animals:

A total number of 215 donkeys belonged to El Kharga city, El-Wadi El-Gadid Governorate were included in this study for the detection of *Strongyle species* worm infection during 2017-2018. Examined donkeys were 125 males and 90 females, while their ages were ranged from one year to 15 years old. Animals were categorized as young (< 3years old), adult (3-8 years) and old (> 8 years). These donkeys were from different locations in El Kharga city (Al-Sherka55, Al-Thawra, Al-Mounira, Al-Osierat, Al-Qattara, Al-Mallaha and Al-Bostan. Examined donkeys had no history of treatment with anthelmintic drugs.

2. Samples:

Fecal samples were collected directly from the rectum of the examined donkeys in a clean plastic cups and the identification number of each animal was registered. Each sample was divided in to two parts, the first part was examined at the same day of collection by floatation sedimentation test for detection of helminths eggs (*Kaplan and Nielsen, 2010*). The second part was used for fecal egg count using McMaster technique (*Presland et al., 2005*). Degree of infection was determined according to number of eggs per gram of feces: < 500 eggs per gram of feces

(EPG) considered to be mild infection, 500 – 1000 EPG is a moderate infection and > 1000 EPG is a severe infection (Soulsby, 1986).

II. Evaluation of drug efficacy.

1. Drugs used in the study:

The following drugs trade marks were used: Ivermectin (Noromectin®1% injectable non- aqueous solution Norbrook co.), dose 0.2 mg/kg Kg body weight injected subcutaneously (s/c). Doramectin (Dectomax®10mg/ml injectable solution Zoetis co.) at a dose of 0.2 mg/kg body weight by s/c injection. Pyrantel (Banminth® Pfizer co. 12.5% pyrantel tartrate) given by oral administration at a dose of 19 mg/kg body weight. Pyrantel dose was calculated, weighted and dissolved in distilled water in order to be administrated orally.

2. Animals used in evaluation of drugs efficacy:

28 donkeys were selected (based on the inclusion criteria) for the drug efficacy experiment. Animals were kept under observation for 3 weeks before starting of the experiment, during which their fecal samples were examined twice per week to confirm single infection with *Strongyle sp.* worms.

Inclusion criteria: Donkeys that agreed with the following criteria were included in the study; had no history of anthelmintic treatment, infected with *Strongyle species* parasite only and showed a minimum fecal egg count of 250 EPG feces or more.

3. Experimental design and methodology:

The 28 *Strongyles* positive donkeys were selectively allocated into 3 treatment groups A, B, C, and one control non-treated group D. each group had mild, moderate and severe infected donkeys which treated with the respective drugs according to the manufacturers' recommendations. The experiment extended for 28 days and fecal samples were collected at day zero before treatment, and at the 7th, 14th, 21st and 28th days post treatment for egg count determination according to *Seri et al. (2005)*. Each animal received a single dose of the selected anthelmintic drug after the first egg count at 0 day as follows:

Group A included 9 donkeys with fecal egg count ranged from 300 to 5850 eggs per gram of feces (EPG) and received ivermectin at a dose 1ml/50 Kg body weight, injected subcutaneous (S/C). Group B included 8 animals with fecal egg count ranged from 250 to 4100 EPG and received doramectin at a dose of 1ml/50Kg body weight by s/c injection. Group C included 6 animals with fecal egg count ranged from 250 – 3750 EPG and received pyrantel by oral administration at a dose of 1g/10Kg body weight for equines. Group D included 5 donkeys with fecal egg count ranged from 450 -1750 EPG and received no treatment as control group. Animal were kept in the stables of Assiut University Veterinary Teaching Hospital

during the study period, and each group was kept in a separate room. Donkeys were observed for possible adverse reactions for 2 hours after medication.

4. Measuring anthelmintic resistance:

a- Fecal egg count reduction test (FECRT) is a technique for testing resistance and was calculated using the following equation (Coles *et al.*, 2006):

$$FECR\% = \frac{\text{pre-treatment EPG} - \text{post treatment EPG}}{\text{pretreatment EPG}} \times 100$$

The post-treatment FEC values were compared with the pre-treatment values, and Arithmetic means (AM) were used. AM is directly proportional to the total egg count of the group of animals. FECR of > 95% for macrocyclic lactones and > 90% for pyrantel is expected for appropriate efficacy (Kaplan and Nielsen, 2010).

b. The FECR should be used in conjunction with the Egg Reappearance Period (ERP) to determine resistance levels effectively. ERP may be a more sensitive early indicator of resistance. ERP Is the time taken for worm eggs to reappear in the feces after de-worming.

III. Statistical analysis.

Data of each individual animals and parasitological examination results were inserted into Ms-excel spread sheet program to create a database. The data were analyzed statistically using the (SPSS statistics 17.0). Differences between parameters (sex, location and age) were tested for significance at probability levels of 0.05 or less. 95% confidence intervals (95% CI) for FECR% were calculated.

Results

Prevalence of Strongyle infection in donkeys:

157 out of the 215 examined donkeys (73.02%) were found to be infected. 93 donkeys out of the infected ones showed signs of emaciation, poor performance, loss of weight, lethargy with anorexia and rough coat, but only 2 cases were having intermittent diarrhea. Infested donkeys were classified into three groups according to degree of infection: first group showed mild degree of infection and represented 35.03% (55) of donkeys, second group showed moderate degree of infection and represented 20.38% (32) of donkeys, while the third group showed severe degree of infection and represented 44.59% (70) of donkeys (Table 1).

a. Sex susceptibility:

Prevalence of *Strongyle species* in female donkeys (75.56%) was slightly higher than in male animals (71.2%), but statistically there were no significant difference ($P = 0.478$; Risk ratio 0.94 [95% Confidence Intervals 0.8 to 1.11]) between infection rate in both sexes (Table 2).

b. Location susceptibility:

Prevalence of strongyle nematode worm showed a highly significant variation according to the location ($P = 0.000$; Chi square = 28.17). The highest prevalence was recorded at Al-Sherka55 (100%) followed by Al-Thawra (84.62%), then Al-Mounira and Al-Osierat (80%), and Al-Qattara (66.67%) and the lowest prevalence was at Al-Mallaha and Al-Bostan where it was 41.18% and 38.46% respectively (Table 2).

c. Age susceptibility:

Donkeys under the age of 3 years old had a slightly higher prevalence (77.42%) than animals from 3 – 8 years old (76.88%). Donkeys more than 8 years old showed no infection rate as showed in (Table 2). Age of the animal had significant effect on prevalence of strongyle worm infection ($P = 0.000$; Chi square = 31.39)

Efficacy of anthelmintics on Strongyle infection in donkeys:

a. Efficacy of ivermectin on Strongyle infection in donkeys:

Mean FECR percentage was 97.05% on day 7 with fecal egg counts 0-650 EPG, and it reached the peak at day 14 (99.6%) and 0 – 50 egg counts range. On day 21 the mean FECR percentage reached 98.3% with a range of 0 – 150 EPG, while on day 28 it reduced to 91.6% and egg count range of 0- 1200 EPG (Table 3).

b. Efficacy of doramectin on Strongyle infection in donkeys:

At day 7 after treatment the fecal egg counts ranged from 0 – 200 EPG and mean FECRT% was 98.6%, while the number of infested animals declined to zero on day 14 after treatment and the mean FECR% reached 100%. Later the egg counts return to increase gradually on days 21 and 28 as it ranged from 0 – 50 and 0-150 EPG respectively and its efficacy slightly decreased to 98.6% on day 21 and 98.2% on day 28 after treatment (Table 4).

c. Efficacy of pyrantel on Strongyle infection in donkeys:

At the 7th day after treatment the fecal egg counts ranged from 50 – 950 EPG with reduction of the mean egg counts by 83.84%. On day 14 fecal egg counts was (0 - 1400 EPG) with mean egg reduction percentage 82.14%, while the mean fecal

count reduction percentage decreased markedly on days 21, and 28 to 78.60% and 67.69% respectively. With increased egg counts range to 0- 1300 EPG at day 21 and 50 – 2250 EPG on day 28. (Table 5).

Table 1. Prevalence of strongyle infection in donkeys and classification of them according to degree of infection.

Number of EPG	Degree of infection	Number	percent
< 500	Mild	55	35.03
500 - 1000	Moderate	32	20.38
> 1000	Severe	70	44.59
Total	215	157	73.02

Table 2. Prevalence of strongyle infection in donkeys based on sex, location and age.

	Examined animals	Positive (%)	Negative (%)	P value
Sex				
Male	125	89 (71.2)	36 (28.8)	0.478
Female	90	68 (75.56)	22 (24.44)	
Total	215	157 (73.02)	58 (26.98)	
Location				
Al -Thawra	39	33 (84.62)	6 (15.38)	0.000
Al-Mounira	25	20 (80)	5 (20)	
Al-Mallaha	17	7 (41.18)	10 (58.82)	
Al-Sherka55	16	16 (100)	0	
Al-Bostan	13	5 (38.46)	8 (61.54)	
Al-Osierat	45	36 (80)	9 (20)	
Al-Qattara	60	40 (66.67)	20 (33.33)	
Total	215	157 (73.02)	58 (26.98)	
Age				
< 3 years	31	24 (77.42)	7 (22.58)	0.000
3-8 years	173	133 (76.88)	40 (23.12)	
> 8 years	11	0	11 (100)	
Total	215	157 (73.02)	58 (26.98)	

Risk ratio (Sex)= 0.94, (95% Confidence Intervals 0.8 to 1.11), Chi square (Location)= 28.17, Chi square (Age) = 31.39.

Table 3. Mean fecal egg counts and mean FECR% for ivermectin treated donkeys (subcutaneous injection).

Day	Arithmetic mean (EPG) \pm SD	Range	Mean Reduction %	95% CI
0	2633.33 \pm 2360.22	300 – 5850	-	-
7 th	77.78 \pm 215.22	0 – 650	97.05	91.9 – 97.8
14 th	11.11 \pm 22.048	0 – 50	99.6	99.1 – 99.9
21 st	44.44 \pm 52.70	0 – 150	98.3	97.1 – 99.6
28 th	222.22 \pm 405.52	0 – 1200	91.6	81.9 – 98.8

Table 4. Mean fecal egg counts and mean FECR % for doramectin treated donkeys (subcutaneous injection).

Day	Arithmetic mean (EPG) \pm SD	Range	Mean Reduction %	95% CI
0	1971.43 \pm 1709.74	250 – 4100	-	-
7 th	28.57 \pm 75.59	0 – 200	98.6	95.9 – 98.9
14 th	0	0	100	99.9 – 100
21 st	28.57 \pm 26.73	0 – 50	98.6	97.6 – 99.5
28 th	35.71 \pm 55.63	0 – 150	98.2	96.3 – 99.9

Table 5. Mean fecal egg counts and mean FECR % for pyrantel treated donkeys (oral administration).

Day	Arithmetic mean (EPG) \pm SD	Range	Mean Reduction %	95% CI
0	1908.33 \pm 1438.89	250 – 3750	-	-
7 th	308.33 \pm 392.96	50 – 950	83.84	69.1 – 98.6
14 th	341.67 \pm 548.10	0 – 1400	82.14	61.5 – 97.3
21 st	408.33 \pm 551.74	0 – 1300	78.60	57.8 – 99.4
28 th	616.67 \pm 909.2121	50 – 2250	67.69	33.5 – 98.1

Table 6. Mean fecal egg counts and FECR % for control non-treated donkeys.

Day	Arithmetic mean (EPG) \pm SD	Range	Mean Reduction %	95% CI
0	850 \pm 525.59	450 – 1750	-	850 \pm 525.59
7 th	1110 \pm 705.69	200 – 1850	-30.59	1110 \pm 705.69
14 th	1170 \pm 797.34	250 – 2200	-37.65	1170 \pm 797.34
21 st	1190 \pm 830.21	450 – 2300	-40	1190 \pm 830.21
28 th	1200 \pm 639.92	500 – 2250	-41.18	1200 \pm 639.92

Discussion

Donkeys represent an important source of traction and income for poor people (Ramaswamy, 1994; Gebreab, 1998). Reviewing the available literature indicated that Strongyle infection appears to be the most prominent parasite that infects donkeys. Strongyles appear to cause many health problems such as colic, profuse diarrhea and un-thriftiness (Soulsby, 1982). According to the authors knowledge, this is the first study to report the prevalence and intensity of Strongyle

worm infection in donkeys in El Kharga city, El Wadi El Gadid Governorate, and the efficacy of the commonly used drugs for treatment.

The overall prevalence of *Strongyle* species infection of donkeys in El Kharga city in El Wadi El Gadid governorate was 73.02% (157/215). This finding was in agreement with that of *Feseha et al. (1991)* and *Tesfu et al. (2014)* who reported 70% and 72.2% in Scotland and Ethiopia respectively. The recorded prevalence was lower than previous reports of *Ahmed (1984)*, *Mohsen (1998)*, *Ayele et al. (2006)*, *Getachew et al. (2010)*; *Ahmed et al. (2011)* and *Bogale et al. (2012)* who recorded 93.80%, 96.38%, 100%, 99%, 81.54% and 82.75% respectively in equines. Meanwhile, it was higher than that recorded by *Seri et al. (2004)*, *Imam et al. (2010)*, *Waqas et al. (2015)*, *Enigidaw et al. (2015)*, *Musa et al. (2016)* and *Osman (2017)* who reported 35.8%, 26.5%, 47.4%, 37.8% and 30.5% respectively. The diversity between our result and those of others may be due to differences in sample size, hygienic status, geographical conditions between study areas and the availability of anthelmintic drugs.

Strongyle worm infection was tested positive in 157 donkeys, 93 of these donkeys were suffering from emaciation, poor performance, weight loss, rough coat, lethargy and anorexia, but only 2 donkeys suffered from intermittent diarrhea. These clinical signs were observed previously by *Umur and Acici (2009)* and *Waqas et al. (2015)*.

The observed clinical signs in the 95 infested donkeys may result from the feeding habits of adult worms, as they feed on mucosal plugs of the intestine of infested host resulting in hemorrhage leading to debility and weight loss or may be due to migration of larvae (*Eckert et al., 2008*; *Osman, 2017*). The remaining 62 donkeys appeared clinically healthy which agreed with the findings of *Urquhart et al. (1996)*, who found that naturally infested animals may tolerate infection if exposed to larvae in small doses over a prolonged period.

The current study reported that 35.03% of the examined donkeys showed mild *Strongyle species* infection, 20% of donkeys showed moderate degree of infection and 44.59% of donkeys had a severe degree of infection. These findings were lower than that of *Wells et al. (1998)* who recorded that all tested positive donkeys were severely infested and were also lower than that of *Getachew et al. (2010)* who indicated that over 55% of the examined donkeys were severely infested by *Strongyle species* as they have fecal egg count more than 1000 EPG. Moreover, this finding agreed with *Upjohn et al. (2010)*, who reported that 48.8% of examined horses were severely infested with FEC over 1000 EPG.

Concerning sex of the examined donkeys, prevalence of *Strongyle species* infection was slightly higher in female animals (75.56%) than in males (71.2%). The difference in prevalence between male and female animals was statistically insignificant ($P = 0.478$), which indicate that sex of the animals has no significant

effect on infection with Strongyle worms. Previous studies reported that males and females' donkeys were equally infested with Strongyle worms, but the slightly increased infection rate in females may be due to their hormonal activities (*Dietz et al., 1984*). Similar findings were recorded by *Ahmed et al. (2011)*, *Sori et al. (2017)* and *Addis et al. (2017)*. In contrast, *Upjohn et al. (2010)* mentioned that female equines are more susceptible to Strongyle infection than male animals, and *Bogale et al. (2012)* obtained that female animals were more susceptible (84.39%) than male animals (83.41%). Also, *Osman (2017)* reported that there was a significant difference between sex of the animals and rate of Strongyle infection as female equines are more susceptible to infection (57.9%) than male animals (25.7%). Meanwhile, *Enigidaw et al. (2015)* recorded that male animals are 1.65 more susceptible to *Strongyle species* infection than females.

Regarding location of the examined donkeys in El-Kharga city, prevalence of Strongyles was 100% at EL-Sherka55; 84.62% at Al-Thawra; 80% at Al-Mounira and Al-Osierat, and 66.67% at Al-Qattara. The lowest prevalence of Strongyle infection was recorded at Al-Mallaha and Al-Bostan as it was 41.18% and 38.46% respectively. This result may be due to the different management methods, poor conditions of housing donkeys, poor nutrition, different workloads and lack of deworming practices between different areas (*Feseha, 1997*).

Regarding age of infested donkeys, our study revealed that infection rate with *Strongyle species* was slightly higher in young animals than in adult and no infection found in old ones. it was 77.42%, 76.88% and 0% in the three age groups respectively. Statistical analysis indicated that the most susceptible age for Strongyle infection was young donkeys < 3 years of age ($P = 0.00$). It may be due to the development of age immunity to Strongyles in adult donkeys (*Chitra et al., 2011*). Lack of immunity against Cyathostome infections could be one reason for greater accumulation of encysted larvae in the intestinal mucosa in young equines (*Herd and Gabel, 1990*). Similar finding was observed previously by *Wells et al. (1998)* and *Addis et al. (2017)*, they recoded that the highest infection rate was at < 3 years old donkeys, followed by donkeys at 3-8 years old and > 8 years (98.7% and 97.4% respectively). *Upjohn et al. (2010)* recoded that the young animals were more susceptible to infection with Strongyle worms than adults. In contrast, *Ahmed et al. (2011)* recorded that old donkeys are more susceptible to infection with large and small Strongyles than young ones.

The main aim of this study was to measure the efficacy of ivermectin and other medications (doramectin and pyrantel) commonly used for treatment of Strongyle worms in El-Kharga city, El Wadi El Gadid governorate. In ivermectin treated group, FEC was reduced by 97.05% on the 7th day, 99.6% on the 14th day, 98.3% on the 21st day, and 91.6% on the 28th day. This finding was nearly similar to that observed previously by *Sipra et al. (1999)*, *Davies and Schwalbach (2000)*, *Imam*

et al. (2010), *Fangama et al. (2013)* and *Zak et al. (2017)*, they mentioned that the reduction in Strongyles FEC was 99.9% at day 14 after treatment with ivermectin. In the present study, although ivermectin showed high reduction percentage in FEC of Strongyles eggs during the first 21 days after treatment, the reduction percentage highly reduced to lower than the 95% resistance limit at the 28th day of treatment. High activity of ivermectin on small Strongyles was reported before, however recently it has been found that small Strongyles eggs began reappearing at 4 weeks which is less than the initial approximate 8 weeks estimated when the drug first was marketed (*Little et al., 2003; Trawford et al., 2005; von Samson-Himmelstjerna et al., 2007*). *Taylor and Kenny (1995)*, *Jacobs et al. (1995)* and *Monahan and Klei (2002)* also reported that egg reappearance period for ivermectin was 8-14 weeks.

Doramectin treated group showed 98.6% FECR on the 7th day, 100% on the 14th day, 98.6% on the 21st day and 98.2% on the 28th day after treatment. This finding was previously observed by *Davies and Schwalbach (2000)* and *Seri et al. (2005)*, they indicated that equines treated by doramectin showed 100% reduction in fecal egg count for 28 days after treatment.

In pyrantel treated group, the mean reduction percentage in Strongyles egg count was 83.84%, 82.14%, 78.60% and 67.69% on the 7th, 14th, 21st and 28th days respectively, after treatment. The mean FECR percentage was less than 90% for the whole 28 days treatment period, and according to (WAAVP) recommendations resistance is found when FECRT% is less than 90%, which indicate resistance of treated animals to the used drug. This result agreed with *Ihler (1996)*, *Craven et al. (1998)*, *Traversa et al. (2007)*, *Molento et al. (2006)*, *Näreahoa et al. (2011)* and *Traversa et al. (2012)*. Under-dosing has been suggested as an important factor in hastening the development of resistance to pyrantel and this is often a risk associated with oral dosing, as spillage frequently occurs (*Luz Pereira et al., 1995*). Our data showed that highest to the lowest ranking of drug efficacy against Strongyles was doramectin, ivermectin and pyrantel. It was shown that donkeys treated with pyrantel drugs had lower EPG values than those treated with doramectin and ivermectin, probably due to the effect of macrocyclic lactones on late luminal larval stages which give a longer suppression of fecal egg output compared with other anthelmintics (*Eysker et al., 1992; Uhlinger, 1992*). These findings may be also attributed to the availability of pyrantel as anthelmintic drug, resistance of the animals to take oral form of this drug, and higher treatment frequency of pyrantel as it considered cheap anthelmintic drugs in Egypt unlike ivermectin and doramectin.

The high activity of ivermectin against small Strongyles, indicated by the data at the first 2 weeks after treatment, may be misleading because EPG counts of these parasites have been returning at about 4 weeks post-treatment. *Lumsden et al.*

(1989) compared the reappearance of nematode eggs in feces after treatment with ivermectin and pyrantel and found that the median period until eggs reappeared in feces was 70 days for ivermectin and 39 days for pyrantel. Based on these results, they suggested a 10-week interval between ivermectin treatments.

Conclusion

This study was the first to report prevalence of *Strongyle* species infection in donkeys in El-Kharga city, El-Wadi El-Gadid governorate, Egypt. Also, it was the first to report ivermectin resistance in infested donkeys in that area. Donkey's owners are recommended to use doramectin for treatment of parasitic infection.

Epidemiološke i terapijske studije o infekciji nematodama magaraca u Egiptu

Maha I. Hamed, Taha A. A. El-Allawy Esraa A. Hassnein

Rezime

Invazija nematodama smatra se glavnim parazitskim problemom kod kopitara, a posebno kod magaraca. Ispitano je ukupno 215 magaraca različitog pola, uzrasta i na različitim lokacijama u gradu El Kharga, guvernoratu El Vadi El Gadid u Egiptu radi otkrivanja infekcije nematodama. Uzorci fecesa su sakupljeni i ispitani pomoću flotacionog testa, a broj jaja je utvrđen McMaster tehnikom. Ukupna prevalenca *Strongyle* vrste iznosila je 73,02% pregledanih magaraca. Od ukupnog broja zaraženih magaraca 35,03% pokazalo je blagu infekciju, 20,38% umerenu infekciju i 44,59% ozbiljnu infekciju. Prevalenca vrste *Strongyle* bila je 75,56% kod ženskih magaraca i 71,2% u mužjaka. Najveća stopa prevalencije zabeležena je u području Al-Sherka 55 (100%), a zatim u području Al-Thavra (84,62%). Životinje mlađe od 3 godine imale su nešto veću prevalencu od životinja 3 - 8 godina, a magarci > 8 godina nisu imali infekciju. Terapeutska efikasnost ivermektina, doramektina i pirantela procenjena je u kontrolisanom eksperimentu u kojem je učestvovalo 28 magaraca koji su prirodno zaraženi crvima vrste *Strongyle*. Doramektin je bio visoko efikasan protiv infekcije glistama vrste *Strongyle* u odnosu na ivermektin i pirantel. U grupi koja je lečena ivermektinom srednji FECR% se 28. dana smanjio na 91.6%. Takođe, otkriveno je da se mali brojevi *Strongyle* EPG vraćaju mnogo brže nego kada je lek prvi put plasiran na

tržište. Prosječni FECR% kod životinja koje su bile tretirane pirantelom bio je manji od 90% tokom 28 dana lečenja. Zaključno, infekcija vrsta *Strongyle* preovladavala je kod magaraca u gubernaturi El-Vadi El-Gadid, a lečenje ivermektinom i pirantelom pokazalo je anthelmintsku otpornost.

Ključne reči: nematode, anthelmintska rezistencija, magarci, Egipat, ivermektin

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SEASON DISTRIBUTION OF GASTROINTESTINAL HELMINTHS OF GOATS KEPT UNDER SEMI- INTENSIVE CONDITIONS IN NORTH WEST SERBIA

Ivan Pavlović¹, Snežana Ivanović¹, Milan P. Petrović², Violeta Caro Petrović²,
Dragana Ružić-Muslić², Nevena Maksimović², Bogdan Cekić²

¹ Scientific Veterinary Institute, Belgrade, Serbia

² Institute for Animal Husbandry, Belgrade, Srbija

Corresponding author: Ivan pavlović, dripavlovic58@gmail.com

Original scientific paper

Abstract: Goat production, in north west Serbia (Mačva district) play important role in providing economical income.. Goats in this region are usually kept under semi-intensive (pasture/stable) condition. One of the main health problems in this kind of production system is connected to infestation with helminths parasites, which results in direct and indirect economical losses. The present study was conducted in 41 goat flocks from the territory of northwest part Serbia in the period of March 2016 to January 2017, using into consideration the seasonal occurrence and prevalence of isolated gastrointestinal parasites. Coprological and post-mortem examination, in a total of 410 fecal samples and 23 post mortem examined goats, revealed the presence of the following gastrointestinal helminths: *Teladorsagia (Ostertagia) circumcincta*, *O.trifurcata*, *O.ostertagi*, *Trichostrongylus colubriformis*, *T. axei*, *Nematodirus spathiger*, *N. filicolis*, *Haemonchus contortus*, *Marshallagia marshalli*, *Bunostomum trigonocephalum*, *Chabertia ovina*, *Oesophagostomum venulosum*, *Cooperia curticei* and *C. oncophora*. The result of this study is a survey of seasonal dynamics of helminth species affecting goats in the region of north west Serbia, also an implication for development of strategic prevention and therapeutic measures.

Key words: goats, endoparasite, north west Serbia, season distribution

Introduction

Today small ruminants, especially goats, play important role in various parts of Serbia in providing animal protein for diet especially for those people who live in the village. At the same time, milk and goat's meat has an increasing demand in the market in cities so that goat's breeding is steadily rising. They

are kept in small herds of 20 to 25 animals. From early spring to late autumn, they are kept on pastures and graze on any land that is not being cultivated (*Ivanović and Pavlović, 2015*).

Pasture breeding make possible contact within small ruminants and eggs, larvae stages and intermediate host of parasites. Those induce that there are no one goat or sheep without parasites. Examination of parasitoses of small ruminants, especially goats were extensive performed at last ten years and we had papers about it in various parts of Serbia. (*Pavlović et al. 2009, 2011, 2012, 2013a,b, 2015a,b, 2017a,b*). In our paper we presented results of parasitology examination of goats in north-west part of Serbia - in Mačva district.

Material and Methods

The study about season distribution of gastrointestinal helminthes of goats at north west parts of Serbia – in Mačva district, was started in March 2016 and finished in January 2017. Mačva is located in the southern edge of Pannonian basin, between the Cer and Fruška Gora Mountains. Territory of Mačva is divided among 3 municipalities: Šabac (including 18 settlements of Mačva), Bogatić (including 14 settlements of Mačva), and Sremska Mitrovica (including 7 settlements of Mačva). Total number of settlements in Mačva is 39, of which 37 are rural, and 2 (Šabac and Mačvanska Mitrovica) are urban. All district is located at 44° 46' northern latitude and 19° 41' east longitude and is in the north-west part of Serbia. Morphologically, the district has three natural zones. North there is a vast plain area known as central Mačva where lowland humus is the dominant soil type. The second morphological unit constitutes the western part of the territory which is characterized by a hilly relief - the Pocerina area, where the plain area Mačva gradually turns into a hilly area down to the Cer Mountain, where the relief and forest caused degradation and evolution of lowland soils into brown forest soil. The third morphological unit covers the southeastern part and pressure occurs in the coldest month of January, while the lowest in April. In this area winds blow from all quadrants. On windless periods as waste 1/3 the frequency of occurrence of winds. The mean air temperature is around 11,7°C. Humidity is on average 78-92% in winter and 51-63% in summer. The annual precipitation is about 435 l/m2. Insolation increases from January to July and then decreases until December (official data by the Hydrometeorological Service of Serbia).

During study we collected fecal samples at monthly intervals. A total of 410 fecal samples originated from 41 flocks were analyzed using standard coprological techniques. A total of 23 we were analyzed by post-mortem examination. Total differential worm counts were performed on all the alimentary tract and lungs using the technique described by *Pavlović and Rogožarski (2017)*.

Determination of adult helminthes and eggs of parasites were done by keys given by *Euzeby (1981)*.

Results and Discussion

The faecal samples were obtained from a different source all together as they were collected from flocks in the field, and the results support the other findings. These counts were also of value in providing some information on the egg rise. The results of necropsy in combination with results of coprological examination, samples appeared to represent the species of parasites adequately.

We revealed same parasite species: *Teladorsagia (Ostertagia) circumcincta* (95.23%), *O.trifurcata* (87.53%), *O.ostertagi* (23.33%), *Trichostrongylus colubriformis* (98.6%), *T. axei* (89.57%), *Nematodirus spathiger* (100%), *N. filicolis* (23.31%), *Haemonchus contortus* (89.95%), *Marshallagia marshalli* (23.77%), *Bunostomum trigonocephalum* (17.28%), *Chabertia ovina* (64.14%), *Oesophagostomum venulosum* (21.39%), *Cooperia curticei* (56.52%) and *C.oncophora* (9.29%).

The intensity of infection and polyparasitism was monitored in relation to the age of goats. It was found that in younger animals intensity of infection was lower than that of older animals. Polyparasitism was observed in all animals. Infection with two species we occurred at 18.13%, with three at 35.86%, four at 22.75%, five at 02.91% and presence of six different nematode genera was detected in 0.52% goats. Adult animals was infected with more parasites species than young and presented potential source of infection and contaminant of pastures.

The ecological parameters had a great influence to the season dynamic of occurrence of parasites species. That condition are the micro and macro climate of the pastures, and these include sunlight, temperature, rainfall, humidity and soil moisture (*Vlassoff, 1982; Pavlović and Ivanović, 2018a*). Second important factors are vegetation and the number of animals on the pasture. Population pressure on the pasture (the number of animals per unit area) and the way of feeding - whether it is protracted or stationary also affects the pasture load and the degree of its infection (*Denev and Kostev, 1994; Familton and McAnulty, 1997; Pavlović et al., 2012, 2013a*). At the beginning of the grazing season on the pastures happily survived the larvae of more resistant species, but shortly afterwards, the pasture is contaminated with parasites infected with the animals that are in it (*Truong et al., 1998; Pavlović et al.2014*). This leads to an increase in the number of species of parasites on both pasture and goats, and this correlation lasts throughout the entire pasture season (*Vlassoff, 1982*). The life cycles of all found helminths species are direct, requiring no intermediate hosts, which applies to all of the economically important strongylid parasites of small ruminants. In these cycles, adult female

parasites in the GI tract produce eggs that are passed out with the faeces of the animal (Theodoropoulos et al, 2000). Development occurs within the faecal mass, the eggs embryonate and hatch from into first-stage larvae (L1), which in turn moult into second-stage larvae (L2) and finally to third-stage larvae (L3) (Skipp et al., 2000). The L3 constitute the infective stage, and these migrate onto surrounding vegetation where they become available for ingestion by grazing sheep and goats (Vlassoff, 1982). The development, survival and transmission of the free-living stages of nematode parasites are influenced by micro-climatic factors within the faecal pellets and herbage (Smith, 1990; Pavlović and Ivanović, 2018b). At climate condition which are present in examined areas, the dynamics of the first occurrence of established species of gastro-intestinal strongilida was as follows:

- In March: *Teladorsagia* (*Ostertagia*) *circumcincta*, *Ostertagia ostertagi*, *Trichostrongylus colubriformis*, *Cooperia oncophora*, *Nematodirus filicoliis* and *N.spathiger*;
- In April: *Ostertagia trifurcata*
- In May: *Trichostrongylus axei*, *Bunostomum trigonocephalum* i *Chabertia ovina*;
- In June: *Cooperia curticei*
- In July: *Haemonchus contortus* and *Oesophagostomum venulosum*;
- In November: *Marshallagia marshalli*

The obtain results confirm that the seasonal distribution of gastrointestinal helminths of goats depends on the microclimate of environmental conditions and that intensity of infection are in correlation with age of animals. Analogous results of influence of animals age to polyparasitism and intensity of infection of helminths we established et small ruminants breed at mountain area of East Serbia including Šara Mountain, Sjenica and Pešter area, and Homolje mountain (Pavlović, 1975; Vujić and Bošković, 1981; Karanfilovski, 1991; Vujić et al., 1991; Ilić et al., 1991; Jovanović et al., 1991; Pavlović et al., 1991, 1995, 2003, 2017a). Similar results were obtained during analogous studies in neighboring Balkan countries Macedonia, Montenegro, Bulgaria and in Romania (Ilijev, 1974; Denev and Kostov, 1984; Georgijevski, 1990, Karanfilovski, 1991; Ardeleanu et al., 2017).

Conclusion

The obtain results confirm that the seasonal distribution of gastrointestinal helminths of small ruminants depends on the microclimate of environmental conditions. However, since the parasitic infections are in majority sub clinical this problem is not played due attention to in our country. The prophylactic treatment is not conducted in the majority of flocks or it is only partially performed what can be seen by the records from the slaughter line and from production results

Sezonska distribucija gastrointestinalnih helminata koza držanih u poluintenzivnim uslovima u severozapadnoj Srbiji

Ivan Pavlović, Snežana Ivanović, Milan P. Petrović, Violeta Caro-Petrović,
Dragan Ružić-Muslić, Nevena Maksimović, Bogdan Cekić

Rezime

Proizvodnja koza u severozapadnoj Srbiji (Mačvanska distrikt) igra važnu ulogu u obezbeđivanju ekonomskih prihoda. Koze u ovom regionu se obično drže u polu-intenzivnom (pašno / stabilnom) držanju. Jedan od glavnih zdravstvenih problema u ovoj vrsti proizvodnog sistema vezan je za infekciju endoparazitima što rezultira direktnim i indirektnim ekonomskim gubicima. Ova studija je sprovedena u 41 stadu koza sa teritorije severozapadnog dela Srbije u periodu od marta 2016. do januara 2017. godine, uzimajući u obzir pojavu i prevalenciju izolovanih gastrointestinalnih parazita. Koprološki i postmortalni pregled, u ukupno 410 uzoraka izmeta i 23 post mortem pregledanih koza, otkrio je prisustvo sledećih gastrointestinalnih helminta: *Teladorsagia (Ostertagia) circumcincta*, *O. trifurcata*, *O. ostertagi*, *Trichostrongylus colubrififormis*, *T. axei*, *Nematodirus spathiger*, *N. filicolis*, *Haemonchus contortus*, *Marshallagia marshalli*, *Bunostomum trigonocephalum*, *Chabertia ovina*, *Oesophagostomum venulosum*, *Cooperia curticei* i *C. oncophora*. Rezultat ove studije je pregled sezone dinamičke vrste helminta koji pogađaju koze u regionu sjeverozapadne Srbije, što takođe implicira razvoj strateške preventivne i terapijskih mera.

Ključne reči koze, endoparaziti, severozapadna Srbija, sezonska distribucija

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INCLUSION SPORE PROBIOTICS «ENZIMSPORIN» IN RATIONS OF SWINES AND ITS EFFECTS ON PRODUCTIVITY, NON-SPECIFIC AND SPECIAL RESISTANCE OF PREGNANT AND LACTATING SOWS

Antonov Valeryi Alekseevich¹, Grishina Marina Anatolievna¹, Nikolaev Sergei Ivanovich², Itskovich Aleksandr Yurievich²

¹Research Institute of Hygiene, Toxicology and Occupational Pathology of the Federal Medical-Biological Agency of Russia, Volgograd, Russia

²Volgograd State Agrarian University, Volgograd, Russia

Corresponding author: Itskovich Aleksandr, itscovic@mail.ru

Original scientific paper

Abstract: Currently studies on the impact of probiotic drugs on productivity and immune system of agricultural animals are updated. In connection with the scientific and practical significance of this problem, studies have been conducted on the use of the probiotic drug «Enzimsporin» in feeding of pregnant and lactating sows and its effect on productivity, nonspecific and specific resistance of sows. In pig breeding bacillary probiotics, due to their high availability, persistence and ease of use are potentially in demand both for biostimulants of growth and for therapeutic purposes. As a result, the preservation of young animals and feed conversion are increasing. The Russian-made drug «Enzimsporin» is a new product based on spore-forming bacteria of the genus *Bacillus*. The main qualities of this probiotic drug are a decrease in the level of colonization of the intestine by conditionally pathogenic microorganisms, the normalization of the intestinal biocenosis and a better assimilation of the nutrients of the feed. Such results are achieving due to: active prevention and correction of stress conditions caused by feed, technological and veterinary factors; prevention of gastro-intestinal diseases; restoration of intestinal microflora from antibiotic therapy and chemotherapeutic drugs; correction of disorders of the digestive processes associated with enzyme deficiency or liver disease; the active replacement of enteropathogenic microflora from the intestinal microbial population. «Enzimsporin» includes a consortium of bacteria of the genus *Bacillus* containing viable spores of at least 5×10^9 CFU/g (colony forming units). Probiotic «Enzimsporin» has no contraindications and has no side effects. Spore probiotic "Enzimsprin" includes the following strains of microorganisms, i.e. their "pure", homogeneous cultures - *Bacillus subtilis* BKM B-2998D (BKIM B-314), *Bacillus licheniformis* BKM B-2999D (BKIM B-8054) and *Bacillus subtilis* BKM

B-3057D (BKIM B-12079). Summary results of experiments can be presented as following - a higher average weight of one pig at weaning and the greatest preservation of the piglets are recorded in sows consumed the probiotic «Enzimsporin» – 5.5 kg and 93.06%, respectively. Animals of the experimental group were superior to the sows of the control group in terms of piglet weight at birth – 1.51 kg to 1.47 kg. The number of stillborn piglets was lower in the experimental group and was 0.61 or 4.76 %. The number of piglets to weaning and therefore their preservation to weaning exceeded those of the control group and amounted to 11.92 heads and 93.06%. The death cases before weaning in the control group was significantly lower (nearly twofold), than in the control group and was at the level of 0.89 heads or 6.94%. The effect of probiotic «Enzimsporin» on the sows' immune system is also confirmed by a higher level of immunoglobulins in the blood serum, which is the most informative indicator of specific resistance. Such an increase in immunoglobulins can be justified by the action of *B. subtilis*, which are part of the probiotic drug.

Keywords: sows, productivity, immune system, spore probiotics, hematological indicators

Introduction

Currently the problems of ecologized agriculture are relevant both in Russia and abroad. Traditional methods of industrial production of livestock products, based on the widespread use of growth stimulants, antibiotics, hormonal and chemical-synthetic veterinary drugs cause serious criticism. This primarily refers to the use of antibiotics and products of their metabolism, as well as hormonal drugs (HD) and their analogues, which are recognized as food pollutants, since, for example, HD promotes the development of diseases such as hypertension, atherosclerosis, coronary heart disease, infertility. In addition, HD violates metabolic processes, reduces resistance and alters the human hormonal status, may have a carcinogenic effect. It has been experimentally proven that hormones stimulate the growth of many types of tumor cells, for example, breast and uterus cancer in women, and prostate cancer in men.

As a result, antibiotics and hormones forming a separate group of food pollutants, the classification feature of which is their use in agriculture and food production. In this regard, recognition of the regulations on the use of antibiotics and hormonal drugs as incorrect is being updated, and their use is an area of increased sanitary and hygienic risks in the management systems of both livestock and processing enterprises. This approach stimulates the formation of the Best

Practices in Livestock, which ensures the production of safe food raw materials (Романов *et al.*, 2010).

Adaptation, survival and reproduction of animals depends on the complex processes of the immune system, which protects the body not only from infectious agents, poisons and polluting chemicals, but also from processes that arise due to stress, damage or abnormal behavior body cells. Any violations of the immune system (including changes in the quantitative and qualitative ratio), undoubtedly, make an organism more susceptible to pathogens of infectious diseases, including conditionally - and lowly pathogenic. In this regard, the search for ways to stimulate the adaptive and innate immune response of animals to the action of stress factors associated with industrial production technology is of great scientific importance. That's why, safe drugs, which include probiotics, are becoming extremely important (Stein, 2005).

The mechanism of action of probiotics works on the principle of forced colonization of the intestine by competitive strains of bacteria - probionts. These probionts exercise nonspecific control over the number of opportunistic microflora, displacing it from the intestinal population. During this process, antibiotic substances are formed, microbial metabolism also changes in the form of a fall or increase in enzyme activity, digestion is normalized, the immune system is stimulated and natural resistance is enhanced (Huang *et al.*, 2008; Lefevre *et al.*, 2015; Ayala *et al.*, 2015; Sebastian and Keerthi, 2014).

With a positive effect in the rations of various animals, probiotic drugs are used, representing cultures of living microorganisms, such as lactobacilli, bifidumbacteria, certain types of bacilli, rumen cellulolytic bacteria and so on (Хорошевский, Афанасьева, 2003). The bacteria *B. subtilis* are one of the most promising probiotics studied in recent decades. The mechanisms of its probiotic action are associated with the synthesis of antimicrobial substances, enhancing nonspecific and specific immunity, stimulating the growth of normal intestinal microflora and secretion of digestive enzymes (Лемяк, 2014; Baruzzi *et al.*, 2011; Stein *et al.*, 2002). In this regard in this work, we studied the effect of the new probiotic drug «Enzimsporin» on productivity and immune system, with a subsequent characterization of its effect on the physiological, reproductive and productive qualities of animals.

The «Enzimsporin» is a powder in minute form that dissolves well in water, which allows to use it through a medicator. It retains its quality in the granulated feed and premixes.

Material and Methods

The aim of the study was to estimate the effect of the probiotic «Enzimsporin» on productivity of sows and changes in blood parameters characterizing the protective

functions of the organism or its immune system. To solve the task, a scientific-industrial experiment was conducted at LLC «TopAgro» of Volgograd region, a large producer of pig products with a population of about 15000 heads of pigs, including 1200 sows.

Experiment involved the F1 sows (Large White x Landrace), which were combined into control and experimental groups, taking into account body weight, age and physiological state. The total number of animals was 360 sows in each group. The conditions of the sows in the experimental and control groups were similar. Feeding animals was carried out according to the scheme adopted in the enterprise. The dosage of «Enzimsporin» supplement in the amount of 50 mg/head per day to the basic ration (BR) for pregnant sows (from 79 to 114 days of pregnancy) and later on to lactating sows (21 days of lactation) was tested. Blood samples were taken from sows of both groups from 6 individuals on fifth day of lactation.

According to scientific-industrial experiment, the following indicators were taken into account – percentage receiving on farrow, the number of piglets at birth, the average live weight of piglets, the preservation of piglets. The second part of experiment was carried out on lactating sows and mainly were taken into account diarrhea cases.

During the study, blood was taken from the ear vein to determine the immunological parameters: quantitative and qualitative ratio of white blood cells, phagocytic number and index, lysozyme, circulating immune complexes and the level of immunoglobulins; physiological parameters of red blood, albumin and total protein in blood serum, erythrocyte sedimentation rate (ESR). The data of laboratory studies correlated with the following indicators of sows productivity—the number of piglets at birth, the piglet weight at birth, the preservation of piglets to weaning, the average live weight of pigs to weaning, diarrhea in piglets.

Phagocytic activity of leukocytes (FAL) and phagocytic index (FI) was established by V.S. Gostevu (1950); serum lysozyme activity - colorimetrically with a culture of *Micrococcus lysodeikticus* cells (I.M. Carpath et al., 1992); bactericidal activity of blood serum - according to O.V. Smirnova and T.A. Kuzmina (1966); the level of circulating immune complexes was determined by spectrometry after processing the tested serum with polyethylene glycol; the level of total immunoglobulins was evaluated in conventional units by the reaction of serum protein turbidity with zinc sulfate by the modified method N.I. Blinov (1985); The level of “natural” antibodies to *Salmonella* V-antigen (hemagglutinins) in the blood of the test animals was judged by the intensity of the hemagglutination reaction. Morphological indices of red and white blood were evaluated on a hematology analyzer ABX PENTRA 60C+; determination of erythrocyte sedimentation rate (ESR) by the Panchenkov method. In the analysis of blood on the hematology analyzer ABX PENTRA 60C +, red blood indicators were evaluated - hemoglobin (HB), erythrocytes (RBC), reticulocytes (RTC), platelets

(PLT), erythrocyte sedimentation rate (ESR), hematocrit (HST), erythrocyte distribution width (RDW)), erythrocyte mean volume (MCV), erythrocyte mean hemoglobin (MCH), erythrocyte mean hemoglobin concentration (MCHC).

Results and Discussion

The impetus for the development of veterinary probiotics was the ban in 2006 of feed antibiotics in the European Union. When using probiotics it is necessary to remember about their quality. Thus, according to the results of studies carried out in the European Union, it is noted that in almost 30% of cases the microorganisms used in probiotic preparations as a valid basis did not correspond to passport data. Often registering the drug and obtaining permission to release it, manufacturing companies reduce the quality of their products due to improper work with strains on breeding, keeping working cultures in an active state, providing conditioned seed materials for production fermentation and others. Work with strains of bacilli is characterized by the complexity of the stable maintenance of their phenotypic characteristics. Evidently quality guarantee of probiotic drugs can be provided only by the leading manufacturers of such products.

Table 1. Total scheme of scientific-industrial testing

Groups	Feeding conditions for sows	Number of sows in experiment, heads
1	Basic ration (BR). Control group	360
2	BR + 50 mg /head of concentrate «Enzimsporin» » per day. Experimental group	360

In the experiment, two groups of animals were formed in according to the principles of the balanced group method, 720 sows were observed, 360 sows (n=360) in each ones. The dosage of «Enzimsporin» in the amount of 50 mg/head per day to the basic ration (BR) for pregnant and later lactating sows was tested. Animals of the experimental group received probiotic «Enzimsporin» through a medicator. In the course of the test, the following indicators were taken into account – percentage receiving on farrow, the number of piglets at birth, the average live weight of piglets, the preservation of piglets. The second part of experiment was carried out on lactating sows. In this test cases of diarrhea were recorded.

Table 2. Scheme of the experiment on pregnant sows from 79 to 114 days of gestation

Groups	Pregnant sows from 79 to 114 days of gestation (360 heads in the group)
Control**	BR**
Experimental*	BR + 50 mg /head of concentrate «Enzimsporin» », watering through the medicator*

**BR - Basic ration (combined concentrate feed)

* Experimental group – use of the medicator

«Enzimsporin» to animals in the experimental group were given from 85 days of gestation and up to weaning on day 21. A higher average weight of one pig at weaning and the greatest preservation of the piglets are recorded in sows consumed the probiotic «Enzimsporin» – 5.5 kg and 93.06%, respectively. Animals of the experimental group were superior to the sows of the control group in terms of piglet weight at birth – 1.51 kg to 1.47 kg.

Table 3. Scheme of the experiment on lactating sows

Groups	Lactation (21 days) (360 heads in the group)
Control**	BR**
Experimental*	BR + 50 mg /head of concentrate «Enzimsporin» », watering through the medicator*

**BR - Basic ration (combined concentrate feed)

* Experimental group – use of the medicator

The number of stillborn piglets was lower in the experimental group and was 0.61 or 4.76 %. The number of piglets to weaning and therefore their preservation to weaning exceeded those of the control group and amounted to 11.92 heads and 93.06%. The death cases before weaning in the control group was significantly lower (nearly twofold) than in the control group and was at the level of 0.89 heads or 6.94%.

Table 4. Productivity of sows during feeding «Enzimsporin»

Indicators	Groups	
	Control (BR)	Experimental BR + 50 mg /head of concentrate «Enzimsporin» »
The number of piglets at birth, heads	12.87±0.05	12.81±0.05
The piglet weight at birth, kg	1.47±0.03	1.51±0.03
The number of stillborn pigs, heads	1.02±0.03	0.61±0.03
The number of stillborn pigs, %	7.92±0.04	4.76±0.04
Death cases before weaning, heads	1.7±0.03	0.89±0.03
Death cases before weaning, %	13.2±0.06	6.94±0.05
Preservation, %	86.8±0.11	93.06±0.09
Taken away piglets, heads	11.17±0.05	11.92±0.06
Average live weight of pigs to weaning, kg	5.4±0.02	5.5±0.02

In practical terms, the use of «Enzimsporin» in an industrial enterprise of pig production is considered from the point of view of reducing diseases such as diarrhea.

Table 5. Effect of feeding probiotic «Enzimsporin» on the incidence of diarrhea piglets

Group/Cause	Number of days 0-7	% 0-7	Number of days 8-14	% 8-14	Number of days 15-21	% 15-21	Number of days >21	% >21	Lost with recorded reasons
Control/ diarrhea	9	28.1	11	34.3	11	34.3	1	3.1	32
Experimental/ diarrhea	0	0	9	50.0	8	44.4	1	5.5	18

The hematological indicators characterizing the state of the red blood sprout corresponded to the normal physiological state, both in the experimental and the control groups, all indicators got within the reference range. Statistically significant differences between groups of examined sows were not found, which is confirmed by the data in Table 6. A study of researches of the white blood sprout in animals of the experimental group allowed to assess the effect of the probiotic drugs on hematological parameters. So statistically significant changes were revealed in the direction of decreasing the absolute and relative number of baton neutrophils and monocytes, as well as an increase in the relative number of eosinophils in sows after taking «Enzimsporin», all the changes in white blood parameters were within the physiological norms. It should be noted that the average values of baton neutrophils and monocytes in the control group of sows

were above the limit of the reference interval.

Table 6. Hematological parameters of sows, n = 6

Investigative indicator	Control group M±m	Experiment group M±m	p from Control	The studied indicator	Control group M±m	Experiment group M±m	p from Control
RBC, 10 ⁶ /μL	5.25±0.19	5.13±0.23	0.695	Baton neu, %	12.50±1.89	5.67±1.28	0.017
HGB, g/dL	107.3±0.34	100.2±0.42	0.216	Neu, %	46.00±0.89	55.00±4.51	0.103
HCT, L/L	32.17±0.98	31.20±1.15	0.538	Eos, %	4.17±0.40	6.50±0.76	0.028
MCV, μm ³	61.33±0.56	61.33±1.17	1.000	Bas, %	0.00±0.00	0.17±0.17	0.363
MCH, pg	20.43±0.15	19.93±0.40	0.288	Mon, %	10.17±0.87	5.67±0.88	0.005
MCHC, g/dL	33.33±0.16	32.57±0.46	0.163	Lym, %	27.17±1.97	27.00±3.41	0.967
RDW, %	16.10±0.33	16.88±0.48	0.213	Baton neu, 10 ³ /μL	1.78±0.28	0.68±0.17	0.010
PLT, 10 ³ /μL	253.33±41.46	227.17±28.12	0.614	Neu, 10 ³ /μL	6.47±0.32	6.73±0.84	0.785
MPV, μm ³	9.40±0.20	9.32±0.32	0.830	Eos, 10 ³ /μL	0.58±0.05	0.88±0.17	0.149
ESR, mm/h	26.67±7.52	13.17±3.96	0.153	Bas, 10 ³ /μL	0.00±0.00	0.02±0.02	0.363
WBC, 10 ³ /μL	14.05±0.54	12.62±1.59	0.426	Mon, 10 ³ /μL	1.41±0.09	0.71±0.13	0.002
				Lym, 10 ³ /μL	3.80±0.27	3.60±0.64	0.782

The results of the analysis of the state of phagocytic part of immunity (PHI is the average number of particles absorbed, phagocytic index (FI is the percentage of cells that entered phagocytosis) of animals of the experimental group did not have statistically significant differences from the phagocytic activity of leukocytes in the control group.

As well as the level of lysozyme, bactericidal activity of serum, "natural" antibodies, circulating immune complexes in serum did not differ from identical indicators of the comparison group. At the same time, a statistically significant increase in the number of immunoglobulins in the serum of sows was detected.

Taking into account that the most stressful period of life in sows falls on the first days after farrowing, the main attention should be paid to the prevention of gastrointestinal diseases, endometritis, mastitis, MMA (mastitis metritis agalactia), diarrhea, increased protective functions of the body, reduced morbidity, increased conversion of nutrients feed into milk production. The changes in the indicators of adaptive and innate immunity discovered in the course of research indicate the stabilization of the sows' immune status after farrowing and the therapeutic and prophylactic effect of «Enzimsporin» probiotic. Thus, in animals of the experimental group, statistically significant differences in the relative and absolute number of baton neutrophils and monocytes within the normal physiological level were revealed. In the control group, which did not receive «Enzimsporin», the average level of these indicators were higher than normal. Monocytes - perform functions similar to those of granulocytes, namely, the capture and destruction of

bacteria and fungi, as well as the recognition and presentation of antigen. Elevated level of monocytes are observed in acute and chronic infectious and inflammatory processes.

Baton neutrophils are a younger form of segmented neutrophils, their increase is often associated with infectious and inflammatory etiology of the pathological condition. The results of the study suggest that this situation indicates the absence of acute infectious-inflammatory process in lactating sows treated with a probiotic, which is confirmed not only by normal indicators of the cellular blood composition, but also by the physiological indicator of ESR (non-specific inflammatory index). In the group of control animals, the average level of erythrocyte sedimentation rate (ESR), on the contrary, was increased by more than 50 %. At the same time, the statistically significant difference revealed in the experimental group in the direction of increasing the relative number of eosinophils (not exceeding the limits of the physiological norm) also indicates the viability of the nonspecific resistance of lactating sows treated with «Enzimsporin».

The effect of probiotic «Enzimsporin» on the sows immune system is also confirmed by a higher level of immunoglobulins in the blood serum (1.5 times higher than in the control group, $p=0.00001$), which is the most informative indicator of specific resistance. The increase in serum immunoglobulins was noted in their studies by other authors. Such an increase in immunoglobulins can be justified by the action of *B. subtilis*, which are part of the probiotic drug. The obtained data are consistent with the results of studies on the biological features of bacilli, which show that *B. subtilis* causes activation and proliferation of B-lymphocytes, which becomes possible due to the release of cytokines from macrophages. In addition, a direct ability to stimulate lymphocytes through cell walls, peptidoglycans and teichoic acids of *B. subtilis* was found. The effect on B-lymphocytes is an increase in the content of immunoglobulins in the blood serum.

Conclusion

According to results it can be concluded that the identified shifts of individual indicators characterizing the action of the immune system of lactating pigs who consumed probiotic are localized within the normal range of adaptive response while maintaining the structural integrity and functional usefulness of all parts of the immune system.

According to experiment results can be presented as following - a higher average weight of one pig at weaning and the greatest preservation of the piglets are recorded in sows consumed the probiotic «Enzimsporin» – 5.5 kg and 93.06%, respectively. Animals of the experimental group were superior to the sows of the control group in terms of piglet weight at birth – 1.51 kg to 1.47 kg. The number of

stillborn piglets was lower in the experimental group and was 0.61 or 4.76 %. The number of piglets to weaning and therefore their preservation to weaning exceeded those of the control group and amounted to 11.92 heads and 93.06%. The death cases before weaning in the control group was significantly lower (nearly twofold), than in the control group and was at the level of 0.89 heads or 6.94%.

The effect of probiotic «Enzimsporin» on the sows' immune system is also confirmed by a higher level of immunoglobulins in the blood serum, which is the most informative indicator of specific resistance. Such an increase in immunoglobulins can be justified by the action of *B. subtilis*, which are part of the probiotic drug.

In total, the action of probiotics is directed to: stimulation of the development of natural immune functions of the body; suppress pathogenic bacteria; treatment of dysbacteriosis and diarrhea; improvement of digestion, break down of acid salts from the bile ducts; reduce cholesterol; synthesize folic acid, biotin, vitamin K and B, niacin; remove toxins from the body. Thus, the use of «Enzimsporin» reduces the incidence of infectious-inflammatory processes after farrowing in lactating sows, which leads to increasing in live weight of pigs, a decrease in the mortality of the litter, as evidenced by the results of the study, which confirms the feasibility of using «Enzimsporin» in pig breeding.

Uključivanje spore probiotika «ensimsporin» u obroke svinja i njihov uticaj na produktivnost, nespecifičnu i specifičnu otpornost suprasnih i krmača u laktaciji

Antonov Valeryi Alekseevich, Grishina Marina Anatolievna, Nikolaev Sergei Ivanovich, Itskovich Aleksandr Yurievich

Rezime

Trenutno se ažuriraju studije o uticaju probiotičkih lekova na produktivnost i imuni sistem farmских životinja. U vezi sa naučnim i praktičnim značajem ovog problema, sprovedena su istraživanja o upotrebi probiotičkog leka «Enzimsporin» u ishrani suprasnih i krmača u laktaciji i njegovom uticaju na produktivnost, nespecifičnu i specifičnu otpornost krmača. Probiotici u svinjarstvu zbog velike dostupnosti, upornosti i lakoće upotrebe potencijalno su u potražnji i kao biostimulansi rasta i u terapijske svrhe. Kao rezultat toga, poboljšano je očuvanje mladih životinja i povećana konverzija hrane. Lek ruske proizvodnje

«Enzimsporin» novi je proizvod zasnovan na bakterijama roda *Bacillus* koje formiraju spore. Glavne osobine ovog probiotičkog leka su smanjenje nivoa kolonizacije creva uslovno patogenim mikroorganizmima, normalizacija crevne biocenoze i bolja asimilacija hranljivih sastojaka hraniva. Takvi rezultati se postižu zahvaljujući: aktivnoj prevenciji i korekciji stresnih uslova uzrokovanih hranljivim, tehnološkim i veterinarskim faktorima; prevencija gastrointestinalnih bolesti; obnavljanje crevne mikroflore od antibiotske terapije i hemoterapijskih lekova; ispravka poremećaja u probavnim procesima povezanim sa nedostatkom enzima ili bolestima jetre; aktivna zamena enteropatogene mikroflore iz crevne mikrobne populacije. «Enzimsporin» uključuje konzorcijum bakterija roda *Bacillus* koji sadrži održive spore od najmanje 5×10^9 CFU/g (jedinice koje formiraju koloniju). Probiotik «Enzimsporin» nema kontraindikacije i nema nuspojave. Spore probiotika «Enzimsporin» uključuje sledeće sojeve mikroorganizama, tj. njihove "čiste", homogene kulture - *Bacillus subtilis* VKM V-2998D (VKPM V-314), *Bacillus licheniformis* VKM V-2999D (VKPM V-8054) i *Bacillus subtilis* VKM V-3057D (VKPM V-12079). Sažeti rezultati eksperimenata mogu se prikazati na sledeći način - veća prosečna težina jedne svinje pri odbijanju i najveća očuvanost prasadi beleže se kod krmača koje su konzumirale probiotik «Enzimsporin» - 5,5 kg, odnosno 93,06%. Životinje eksperimentalne grupe bile su superiorne u odnosu na krmače kontrolne grupe u pogledu težine prasadi na rođenju - 1,51 kg do 1,47 kg. Broj mrtvorodne prasadi bio je manji u eksperimentalnoj grupi i iznosio je 0,61 ili 4,76%. Broj prasadi za odbijanje i stoga njihovo čuvanje do zalučenja premašio je broj kontrolne grupe i iznosio je 11,92 grla i 93,06%. Slučajevi smrti pre odbijanja u kontrolnoj grupi bili su značajno niži (gotovo dvostruko), nego u kontrolnoj grupi i bili su na nivou od 0,89 grla ili 6,94%. Dejstvo probiotika ««Enzimsporin» » na imuni sistem krmača potvrđuje i viši nivo imunoglobulina u serumu u krvi, što je najinformativniji pokazatelj specifične otpornosti. Takvo povećanje imunoglobulina može se opravdati delovanjem *B. subtilis*, koji su deo probiotičkog leka.

Ključne reči: krmače, produktivnost, imuni sistem, probiotici, hematološki indikatori

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THE INFLUENCE OF AGE AND BREED OF PIGS ON THE CONTENT OF TOTAL AND SOLUBLE INTRAMUSCULAR COLLAGEN

Lukasz Migdal¹, Krzysztof Krzysztoforski³, Anna Migdal²,
Wladyslaw Migdal³

¹Department of Genetics and Animal Breeding

²Institute of Veterinary Science, Faculty of Animal Science, University of Agriculture in Krakow, al. Mickiewicza 24/28, 30-059 Kraków, Poland

³Department of Animal Product Technology, Faculty of Food Technology, University of Agriculture in Krakow, ul. Balicka 122, 31-149 Kraków, Poland

Corresponding author: Lukasz Migdal, lukasz.migdal@urk.edu.pl

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Abstract: Gilts of Polish Landrace, Polish Large White, Pulawska (Polish native breed), Duroc and Pietrain breed were slaughtered at 60, 90, 120, 150, 180 and 210 days of age (six animals in each age group). The content of total and soluble collagen of gilts was measured in *m. longissimus dorsi* (LD) and *m. semimembranosus* (SM). Breed had no effect on collagen content but age of gilts had a significant impact on collagen content *m. longissimus dorsi* (LD) had higher content of collagen than *m. semimembranosus* (SM). In high carcass leanness' breed (Pietrain) soluble collagen content increased with age while in low carcass leanness' breed (Pulawska breed) soluble collagen content decreased with age. It was also stated that the roasted meat had higher collagen content than the raw meat. Favourable characteristic of tenderness (more tender) between analysed breed of pigs was found in Duroc breed.

Keywords: pigs, collagen, age, breed, shear force

Introduction

Tenderness is considered as the basic quality component of culinary meat. Among parameters influencing the meat tenderness determined by organoleptic or shearometric methods (shear force and penetration measurements), characteristics of muscle fibre and connective tissue are referred mainly. Commonly pork meat is included to tender meat however, recent research shows that there is a quite considerable variation of this trait, associated with increase of fleshiness in pigs (Wood *et al.*, 1986; Cameron *et al.*, 1990; Szalata

et al., 1999; Wood *et al.*, 2004). Increase of meat content in carcass very often leads to deterioration of tenderness. This is most likely associated with a lower intensity of protein degradation processes during post-mortem ageing in high carcass leanness' breed (Szalata *et al.*, 1999). The most important role in meat tendering process has a fraction of connective tissue proteins, including levels and forms of collagen. The hardness of the meat is related to the structures of strong inextensible fibers around all muscle formed by collagen. About 90% of intramuscular collagen can be found in *perimysium*, and its total content in dry mass of meat is between 2 – 6% Palka (1995). In intramuscular connective tissue few types of collagen were identified – e.g. I, III, IV, XII, XIV collagens (Listart *et al.*, 2000). The important characteristic of collagen is its insolubility in conditions not affecting its natural structure. Using solutions with varying pH and ionic strength, as well as initial mechanical, chemical or enzymatic treatment, it is possible, as result of selective destruction of cross – linking bonds, to get almost completely soluble collagen (Krasnowska, 1998). With age, structure of collagen of animals of slaughters is changing, becoming more compact and its solubility decreases (Light *et al.*, 1985; Kolczak *et al.*, 1992; Correa *et al.*, 2006). Kolczak *et al.*, (1992) found significant positive correlation between solubility of collagen and tenderness of meats of animals in different age, which was sensory evaluated. The objective of the study was to determine how age and breed of pig influence on solubility of intramuscular collagen and on shear force of raw and roasted meat

Materials and Methods

A total of 180 gilts (RYRI -- free) from five breeds: Polish Landrace (PL), Polish Large White (PLW), Duroc (D), Pietrain (PI) and Pulawska (PU) were used in this study (36 gilts of each breed). Gilts were kept from 60 to 210 days of age at the Pig Testing Station in Pawłowice (Poland) and fed a complete diet. All animals were reared under the same environmental and production regime. Pigs of each breed were slaughtered at 60, 90, 120, 150, 180 and 210 days of age (6 sows of each age from each breed) in commercial slaughterhouse. Feed was withdrawn 12 h before slaughter but water was freely available. The pigs were stunned with CO₂ and processed according to the normal slaughterhouse procedures (exsanguinated, scalded, dehaired and eviscerated). After 24 h of meat ageing from half carcass samples of *m. longissimus dorsi* (LD) and *m. semimembranosus* (SM) were collected. Meat was cut into slices: half were further analysed as raw meat another half were wrapped with aluminium foil and roasted in electric oven in 180°C until it reached 74 °C in geometric centre of meat slice. The temperature was

measured with needle probe thermometer. After obtaining desired temperature, slices of meat were cooled to room temperature. From the raw and roasted slices of LD and SM samples for shear force measurement were cut out. The determination of total and soluble collagen content was performed on raw and roasted muscles samples.

Total collagen content

The total collagen content was estimated according to Polish Norm (*PrPN-ISO 3496*). About 4 g of minced meat was mineralised in 30 ml 3M H₂SO₄ in pyrex tube. Then the tube was capsulated with teflon and hydrolysed in 105°C during 16 h. Hot hydrolysate was filtered to 250 ml flask and diluted with distilled water. In the diluted hydrolysate the hydroxyproline content was estimated. 4 ml of diluted hydrolysate was placed in 15 ml ground neck flask, 2 ml of T chloramine added. Then the colour reaction with 2 ml of DABA (4--(dimethylamino – benzaldehyde) was evoked. Sealed ground neck flasks were heated during 20 min. in water bath in 60°C, then cooled for 3 min. in cold water and left for 30 min. in room temperature. The absorbance of samples was measured with Novasina spectrophotometer at 558 nm. The hydroxyproline content was then read from calibration curve. The total collagen content was calculated from hydroxyproline amount with 7.25 index and with dilution factors included.

Content soluble collagen

The soluble collagen was calculated as a difference between total amount of collagen and not soluble collagen. The amount of not soluble collagen was estimated according to Liu et al. (1994) method with own modification. To 6 g of minced meat 24 ml tree times diluted Ringer solution was added and the sample was then homogenised with MPW-120 homogenizer during 1 min. at 10000 rpm/min. The homogenate was heated in 77°C in water bath for 70 min. and then centrifuged at 3500 rpm/min. during 20 min. The solid phase was then washed up with 24 ml of diluted Ringer solution and once again centrifuged. The solid phase was dried in 105°C air dryer to stabilize weight and crushed in a mortar. 100 mg of crushed powder was hydrolised with 3M H₂SO₄ and procedure was repeated as for total collagen. The hydroxyproline content was read from spectrophotometric curve. Not soluble collagen content was calculated with 7.25 index. The amount of soluble collagen was calculated and expressed as percentages of total collagen.

Shear force

The measurements of shear force were performed for each pigs' carcass, at 7 samples cut out from roasted and cooled to room temperature muscle. The sample of 15 mm height and 16 mm diameter were cut out parallel to muscle fibres. The shear force was measured with TA-XT2 Texture Analyser (Stable Micro Systems, England) and Warner – Bratzler blade. The Warner—Bratzler blade measuring speed was 2 mm/s, using a force of 10 g. The data for shear force of assessed samples were analysed with Texture Exponent 32 software. The results were analysed statistically using Statistica for Windows 8.0, calculating arithmetic average and standard deviation. Influence of breed, age of fatteners and roasting on changes of total content and solubility of intramuscular collage were evaluated using three-way ANOVA according to the model:

$$Y_{ijk} = \mu + \alpha_i + \beta_j + \gamma_k + e_{ijk}$$

μ – overall mean, α – effect of breed of pig (5 breeds), β – effect of age (6 age spans), γ – muscle effect (2 muscles), e – residual error.

Results and Discussion

In Table 1 the total collagen content was showed whereas in Table 2 the content of soluble collagen defined as % of total collagen in raw pig meat depending on breed and age. In LD muscle total collagen content was between 0.60 (PLW) – 1.19% (PU) at 60 days of age and 0.42 (PI) – 0.87% (PLW) in 210 days of age. Total collagen content in SM muscle in 60 day of age ranged from 0.49 (PLW) to 0.85% (D) whereas in 210 days of age from 0.44 (PL) to 0.59% (PU). The results for analyses of variance presented in table 5 showed that breed has not affected total collagen content, however, age of gilts has highly significant influence. In LD muscle higher total collagen content was found in comparison to SM muscle (Table 5). At 60th day of age in LD muscle the highest total collagen content was found in PL, D, PI, PU breed, whereas at 210th day of age in PL, PI, PU breed total collagen content was the lowest in the same muscle. In PLW the lowest collagen content in LD muscle was observed in 60th and 120th days of age while the highest content was observed in 90, 180 and 210 days of age.

Table 1. Total collagen content (%) in raw muscles of pigs depending on the breed and age ($\bar{x} \pm SE$)

Breed	Muscle	Age of animals (days)					
		60	90	120	150	180	210
Polish Landrace	LD	1.05±0.21	0.84±0.25	0.70±0.17	0.53±0.17	0.67±0.32	0.44±0.09
	SM	0.49±0.05	0.71±0.08	0.59±0.14	0.84±0.18	0.77±0.39	0.44±0.09
Polish Large White	LD	0.60±0.53	0.94±0.46	0.59±0.19	0.79±0.52	0.88±0.43	0.87±0.46
	SM	0.52±0.28	0.67±0.20	0.59±0.13	0.65±0.22	0.56±0.15	0.57±0.12
Pietrain	LD	1.01±0.35	0.53±0.28	0.75±0.20	0.58±0.15	0.57±0.29	0.42±0.12
	SM	0.79±0.26	0.63±0.24	0.77±0.57	0.45±0.14	0.54±0.30	0.51±0.14
Duroc	LD	1.02±0.14	0.55±0.10	0.81±0.05	0.65±0.18	0.58±0.19	0.79±0.57
	SM	0.85±0.27	0.54±0.09	0.48±0.09	0.56±0.11	0.69±0.01	0.46±0.08
Pulawsk ^a	LD	1.19±0.38	0.72±0.35	0.74±0.22	0.64±0.41	0.56±0.17	0.52±0.18
	SM	0.67±0.23	0.60±0.18	0.94±0.59	0.65±0.27	0.56±0.19	0.59±0.19

LD - *m. longissimus dorsi*

SM - *m. semimembranosus*

Table 2. Soluble collagen content (% of total collagen) in raw muscles of pigs depending on the breed and age ($\bar{x} \pm SE$)

Breed	Muscle	Age of animals (days)					
		60	90	120	150	180	210
Polish Landrace	LD	3.45±1.45	5.97±3.01	3.03±1.10	3.28±0.81	3.76±1.79	4.76±2.97
	SM	7.73±0.20	6.06±1.35	1.98±0.32	3.68±1.04	5.54±4.19	3.71±1.37
Polish Large White	LD	3.95±1.99	6.07±3.19	2.03±0.74	2.35±1.31	4.26±2.65	4.64±2.14
	SM	6.25±4.03	4.60±3.05	2.60±0.73	3.17±1.91	4.48±2.22	3.92±2.14
Pietrain	LD	5.18±2.95	4.54±2.43	7.73±2.98	7.17±3.01	9.58±8.18	7.52±3.49
	SM	7.76±3.60	8.15±3.83	5.38±3.65	8.86±5.04	5.38±1.35	9.41±3.21
Duroc	LD	8.11±3.27	8.70±5.53	4.77±1.70	7.12±3.76	7.67±3.52	6.39±2.99
	SM	7.70±1.78	3.37±3.25	4.78±1.47	8.27±4.07	8.31±5.51	7.97±3.93
Pulawska	LD	6.39±2.51	6.69±2.91	3.29±1.82	2.46±1.38	4.37±2.09	3.73±2.70
	SM	4.44±1.98	4.50±2.06	4.37±2.60	4.80±2.99	3.40±2.22	2.79±1.63

LD - *m. longissimus dorsi*

SM - *m. semimembranosus*

In Table 3 the content of total collagen was showed. In table 4 the content of soluble collagen (as % of total collagen) in roasted pig muscles depending on breed and age was presented. The content of soluble collagen in LD muscle at 60 days of age of pigs was between 9.49% of the total content (PL) and 18.48% (PU), whereas in 210 days of age from 6.26% (PU) to 11.91% (PL). In roasted SM muscle content of soluble collagen was significantly higher (11.83% of total collagen) compared to LD muscle (7.54% of total collagen). In raw muscles soluble collagen content was similar. In our study, we found that with age content of soluble collagen increases in high carcass leanness'

breed (PI), whereas the content of soluble collagen decreases in low carcass leanness' breed (PU). The analysis of variance of soluble collagen in raw meat (Table 5) showed the influence of age and breed of pigs on the content of soluble collagen ($p < 0.05$). In raw muscles from Pietrain and Duroc breed the content of soluble collagen was significantly higher compared to Pulawska, Polish Landrace and Polish Large White breed. In roasted muscles higher content of soluble collagen was found in Pietrain, Polish Landrace and Polish Large White breed. With age of pigs content of soluble collagen was decreasing both in raw and roasted muscles. We observed that in roasted muscles content of collagen was higher then in raw muscles. This correlation was caused by surface evaporation and thermal drip loss. Similar relationships were showed by *Kolczak et al. (2003)* in beef meat. Moreover, *Kolczak et al. (1992)* stated that with cattle maturity structure of collagen becomes more compact and its solubility is decreasing. Along with the aging of collagen, its structure is stabilized by cross – linking inner – and intermolecular covalent type bonds. These bonds have highest influence on collagen solubility (*Krasnowska, 1998*). *Bailey and Light (1989)* found that with maturity of animals, solubility of collagen decreases and is positively correlated with meat tenderness. During the instrumental analysis of shear force of roasted muscles from Polish Landrace, Polish Large White, Duroc, Pietrain and Pulawska breed it was observed that shear force of SM muscles was higher then of LD – Tables 6 and 7. It is expected that the content of collagen does not affect meat tenderness but the tenderness is influenced by its intermolecular cross – linking formation in collagen, which depends on physiological maturation. *Coró et al. (1999)* suggested that higher content of collagen in poultry may decrease meat tenderness. *Chelmicka (2000)* found that differences in meat tenderness at the same collagen content may be caused by different structure of collagen fibres. Thicker collagen fibres may worsen meat tenderness and this can be observed in young animals despite the fact that the influence of collagen on tenderness is not significant statistically (*Pospiech et al., 2003*).

Table 3. Total collagen content (%) in roasted muscles of pigs depending on the breed and age ($\bar{X} \pm SE$)

Breed	Muscle	Age of animals (days)					
		60	90	120	150	180	210
Polish Landrace	LD	0.82±0.12	0.83±0.35	0.66±0.14	1.18±0.91	0.62±0.06	0.68±0.41
	SM	0.86±0.15	0.82±0.11	0.99±0.17	0.58±0.13	0.74±0.12	0.65±0.12
Polish Large White	LD	0.79±0.27	0.85±0.29	0.82±0.16	0.78±0.29	0.85±0.31	0.61±0.10
	SM	0.85±0.16	1.09±0.34	0.64±0.15	0.83±0.23	0.80±0.35	0.79±0.40
Pietrain	LD	0.93±0.21	1.08±0.40	0.98±0.23	0.63±0.11	1.00±0.64	0.50±0.13
	SM	0.82±0.18	0.68±0.06	0.64±0.14	0.80±0.22	0.66±0.11	0.73±0.19
Duroc	LD	1.09±0.49	0.91±0.12	0.65±0.01	0.71±0.15	0.45±0.09	0.69±0.34
	SM	0.78±0.20	0.79±0.13	0.85±0.24	0.83±0.31	0.65±0.10	0.66±0.22
Pulawska	LD	1.04±0.32	0.78±0.15	0.67±0.16	0.69±0.10	0.80±0.36	0.69±0.31
	SM	1.01±0.22	0.87±0.20	0.68±0.20	0.63±0.14	0.65±0.14	0.84±0.32

LD - *m. longissimus dorsi*

SM - *m. semimembranosus*

Table 4. Soluble collagen content (% of total collagen) in roasted muscles of pigs depending on the breed and age ($\bar{X} \pm SE$)

Breed	Muscle	Age of animals (days)					
		60	90	120	150	180	210
Polish Landrace	LD	9.49±2.04	11.00±0.46	18.6±1.06	15.96±6.59	11.17±0.48	11.91±5.48
	SM	7.59±1.81	9.05±1.24	14.03±0.92	6.90±2.95	4.80±0.24	6.30±2.25
Polish Large White	LD	12.82±2.46	13.08±6.78	10.19±1.87	12.49±5.80	10.23±8.38	11.54±5.09
	SM	10.73±1.66	7.93±3.35	8.30±2.13	10.79±5.22	6.66±3.20	9.35±6.83
Pietrain	LD	12.26±4.09	13.26±5.31	18.93±5.17	11.92±9.32	16.16±9.11	9.86±9.62
	SM	11.61±3.22	8.27±1.15	6.80±2.64	5.22±3.57	7.39±1.48	4.10±1.52
Duroc	LD	15.44±4.51	7.84±1.34	8.17±1.70	10.07±6.59	7.52±4.68	10.59±5.99
	SM	11.86±3.80	7.20±3.01	5.98±4.03	6.85±2.12	3.98±0.59	5.24±1.74
Pulawska	LD	18.48±8.28	11.43±7.32	9.48±4.94	8.81±3.88	10.59±4.00	6.26±1.79
	SM	6.37±3.07	4.73±0.90	7.42±2.59	6.28±1.90	8.42±2.73	6.11±4.69

LD - *m. longissimus dorsi*

SM - *m. semimembranosus*

Table 5. Least squares analysis of variance

Muscle	Collagen	Breed of animals					Type of muscle		Age of animal (days)					
		Polish Landrace	Polish Large White	Pietrain	Duroc	Pulawska	LD	SM	60	90	120	150	180	210
n		36	36	36	36	36	90	90	30	30	30	30	30	30
Raw	Total	0.67	0.68	0.62	0.66	0.62	0.71 ^a	0.62 ^b	0.81 ^a	0.67 ^{bc}	0.69 ^b	0.63 ^{bc}	0.63 ^{bc}	0.56 ^c
	Soluble	4.41 ^a	4.02 ^a	7.22 ^b	6.92 ^b	4.26 ^a	5.29	5.44	6.09 ^a	5.84 ^a	3.99 ^b	5.11 ^{ab}	5.67 ^a	5.48 ^a
Roasted	Total	0.78	0.80	0.78	0.75	0.77	0.79	0.77	0.90 ^a	0.87 ^{ac}	0.75 ^b	0.76 ^{bc}	0.72 ^b	0.68 ^b
	Soluble	10.52 ^a	10.34 ^a	10.48 ^a	8.39 ^b	8.69 ^{ab}	7.54 ^a	11.83 ^b	11.66 ^a	9.34 ^{bc}	10.73 ^{ac}	9.52 ^{bc}	8.69 ^b	8.12 ^b

LD - *m. longissimus dorsi*

SM – *m. semimembranosus*

Values in the same rows with different letters differ significantly a, b, c - $P \leq 0.05$,

Texture of meat, including tenderness, depends on muscle fibres diameter, amount of fibres, and amount and thickness of connective tissues bands (Bailey and Light 1989). Wojtysiak *et al.* (2010) analysing muscle fibres of SM fatteners of Polish Landrace, Polish Large White, Duroc, Pietrain and Pulawska breed slaughtered at 60, 90, 120, 150, 180 and 210 days of life found that in all analysed breeds, the highest increase in muscle fibre diameter was present in pigs aged between 60 and 120 days. After 150 days of breeding the rate of muscle fibre growth was slower and was not significant between 180 and 210 days. Among analysed breeds of pigs, favourable tenderness parameters were found in meat from Duroc breed, similar to Florowski *et al.* (2006). Meat from Duroc breed has high content of intramuscular fat which lead to smaller shear force and compression and also very good culinary usefulness. Although collagen stands only for 2% of total amount of proteins in muscle, it is mainly responsible for muscle textural changes during heating. Because collagen undergoes structural denaturation and solubilisation (Powell *et al.*, 2000), so its partial solubility decides about meat tenderness (Bailey and Light, 1989).

Table 6. Hardness [N] value of roasted loin (*m. longissimus*) and ham (*m. semimembranosus*) fatteners' depending on the breed and age ($\bar{x} \pm SE$)

Breed	Muscle	Age of animals (days)					
		60	90	120	150	180	210
Polish Landrace	LD	58.23±15.19	35.53±17.96	88.73±17.91	113.14±19.85	125.21±12.75	124.63±13.67
	SM	85.57±19.30	75.89±35.63	113.21±22.73	119.9±21.78	95.11±8.74	132.51±28.27
Polish Large White	LD	57.20±8.59	69.56±7.86	107.94±29.19	100.17±29.46	92.60±7.46	101.85±25.84
	SM	87.56±33.49	101.11±18.31	107.07±19.58	110.13±40.87	101.73±41.10	95.88±15.57
Pietrain	LD	61.74±19.93	63.09±11.76	95.64±14.67	87.11±11.43	104.94±30.09	122.46±36.85
	SM	90.39±23.29	76.99±13.34	120.04±35.26	111.84±40.88	112.91±8.83	117.20±21.01
Duroc	LD	54.45±19.81	61.18±17.48	79.50±12.95	81.22±19.42	80.77±21.60	89.25±19.11
	SM	100.78±37.48	94.01±10.83	104.42±16.31	104.09±22.64	89.39±17.77	109.46±13.53
Pulawska	LD	62.17±10.86	58.86±10.57	90.32±24.25	111.20±26.60	99.53±22.85	100.91±29.24
	SM	82.79±28.21	89.44±6.71	120.62±17.45	106.83±29.54	101.03±23.51	103.80±17.48

Table 7. Least squares analysis of variance of roasted meat

Type of muscle	Breed of animals					Age of animals (days)					
	Polish Landrace	Polish Large White	Pietrain	Duroc	Pulawska	60	90	120	150	180	210
n	36	36	36	36	36	30	30	30	30	30	30
LD	93.64 ^a	88.94 ^{ab}	87.97 ^{ab}	74.79 ^{bp}	86.83 ^{ab}	59.04 ^{Ap}	57.82 ^{Ap}	92.09 ^{Bp}	99.04 ^B	101.34 ^B	108.32 ^B
SM	105.87	100.33	103.78	100.7 ^r	100.26	89.28 ^{ar}	87.15 ^{ar}	113.09 ^{br}	110.65 ^h	99.88 ^{ab}	112.13 ^b

LD – *m. longissimus dorsi*

SM – *m. semimembranosus*

Values in the same rows with different letters differ significantly - a, b, c - $P \leq 0.05$

A, B - $P \leq 0.01$

Values in the same columns rows with different letters differ significantly - p, r - $P \leq 0.05$

Conclusions

In conclusion, it may be stated that meat tenderness therein shear force depends more on quality of connective tissue than on amount of connective tissue. Shearometrically tenderness depends on collagen types, fibre and bond of collagen fibres and thermal stability of intermolecular cross – linkage.

Uticaj starosti i rase svinja na sadržaj ukupnog i rastvorljivog intramuskularnog kolagena

Lukasz Migdał, Krzysztof Krzysztoforski, Anna Migdał, Władysław Migdał

Rezime

Nazimice poljskog landrasa, poljskog jorkšira, pulavske (poljska autohtona rasa - Pulawska), durok i pijetren zaklane su u uzrastu od 60, 90, 120, 150, 180 i 210 dana (po šest životinja u svakoj starosnoj grupi). Sadržaj ukupnog i rastvorljivog kolagena nazimica izmeren je u *m. longissimus dorsi* (LD) i *m. semimembranosus* (SM). Rasa nije imala uticaj na sadržaj kolagena, ali starost nazimica je imala značajan uticaj na sadržaj kolagena *m. longissimus dorsi* (LD) imao je veći sadržaj kolagena od *m. semimembranosus* (SM). Sadržaj rastvorljivog kolagena u nazimicama rase koja se odlikuje izrazitom mesnatošću trupa (pijetren) povećavao se s uzrastom, dok se u nazimicama rase sa slabom mesnatošću (Pulavska rasa) sadržaj rastvorljivog kolagena smanjuje s uzrastom. Takođe je navedeno da pečeno meso ima veći sadržaj kolagena u odnosu na sirovo meso. Povoljna karakteristika mekoće (mekše meso) između analiziranih rasa svinja utvrđena je kod rase durok.

Ključne reči: svinje, kolagen, starost, rasa, sila presecanja

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POSIBILITIES FOR UTILIZATION OF CARBON DIOXIDE FROM POULTRY IN GREENHOUSE PLANTED LETTUCE (*LACTUCA SATIVA*)

Ivan Yanchev, Kamelia Kancheva

Institute of Animal Science, 2232 - Kostinbrod, Bulgaria
Corresponding author: Ivan Yanchev, ijantcev@mail.bg
Original scientific paper

Abstract: The aim of the present study was to evaluate the effect of increased CO₂ levels as a compound of mixed emissions from a poultry housing (transported by an air duct into a greenhouse) on lettuce' growth and its content. The experiment was carried out at the experimental poultry farm of the Institute of Animal Science - Kostinbrod in May and June, 2019 for 40 days. During the experiment the following parameters were controlled and measured both in control and experimental greenhouses (lettuce groups): average level of CO₂, indoor air temperature and relative humidity, average level of ammonia (NH₃), average total moisture/dry matter, crude protein and ash, Ca and P content, Vegetative biomass index (NDVI) and an average weight of lettuce at 1-st, 17-th and 40-th day. The obtained results showed that lettuce plants in the experimental greenhouse had significantly higher crude protein content ($p < 0.01$) during the experiment than those in control group, which can be related to the increased CO₂ content. There were no significant differences at average weight of the plants in both groups, but they had a trend of a different growth rate during the experiment, possibly due to the mixed and variable emission from the poultry house. The total moisture, Ca and P content and the NDVI in both groups were similar.

Key words: poultry, carbon dioxide, ammonia, greenhouse, lettuce, protein

Introduction

Emissions of CO₂ from livestock in closed buildings with forced ventilation can be estimated quite accurately. Except emissions by combustion of natural gas for heating, which are approximately constant, the main source of CO₂ in poultry is respiration rate and decomposition of organic matter depending on technology used (*Knížatová et al., 2010*). The level of protein and other nutritional factors also provide increased metabolism and production of CO₂ (*Mihina et al., 2012*). Carbon

dioxide production by broilers is proportional not only to their number but also to their metabolic heat production, and thus to the metabolic body weight of the broilers, which in turn is affected by the temperature and their activity (*Vučemilo et al., 2007*). Some authors reported that the CO₂ production under “normal farm conditions” in poultry has normally a daily variation of $\pm 20\%$, which can be related to different “personnel conditioned activity” around-the-clock (*Pedersen et al., 2008*). *Calvet et al. (2011)* calculated different average CO₂ emission rates per poultry in summer and winter - 3.84 and 4.06 g.h⁻¹, respectively.

On the other hand the benefits of carbon dioxide supplementation on plant growth and production within the greenhouse environment have been well investigated for many years (*Makino and Mae, 1999*). Most crops show that for any given level of photosynthetically active radiation (PAR), increasing the CO₂ level to 1,000 ppm will increase the photosynthesis by about 50% over ambient CO₂ levels. In general, as reviewed by *Prior et al. (2011)*, elevated CO₂ increases plant growth (both above-and belowground) and improves plant water relations (reduces transpiration and increases water use efficiency).

Consequently the aim of the present study was to evaluate the effect of increased CO₂ levels as a compound of mixed emissions from a poultry housing (transported by an air duct into a greenhouse) on growth, yield and quality parameters of lettuce.

Material and Methods

The experiment was carried out at the experimental poultry farm of the Institute of Animal Science - Kostinbrod in May and June, 2019 for 40 days. In the barn (12 m wide, 45 m length and 3,50 m height) 1800 laying hens Cobb 500 (Cobb) and Lohmann-Brown Classic (LBC) at age 10 months were reared at the beginning and 750 at the end of experiment. The litter was constant and the ventilation was through 8 electric fans Ø500 mm with capacity of 7200 m³/h, which automatically have been turning on and off at inside temperature of 18 °C.

From one of the fans, an aluminum foil air duct Ø110 was connected providing 1584 m³/h air flow into near 12m³ polyethylene experimental greenhouse (area of 6 m²) – Picture 1. The same control polyethylene greenhouse (Picture 2) was situated near the experimental one and the humus layer in both greenhouses was identical. Three windows (0.75 m²) stayed opened during the whole experiment in both greenhouses.



Picture 1. Experimental greenhouse



Picture 2. Control greenhouse

The agrochemical indicators of soil analysis (Table 1) showed a high potassium content, humus 1.27% and nitrogen - 11.5 mg kg^{-1} . The samples were

analyzed in the authorized Central laboratory of the Institute of Soil Science “Nikola Poushkarov” - Sofia by standard methods with a certificate.

Table 1: Agrochemical indicators of soil analysis

pH		$\Sigma \text{N-NH}_4 + \text{NO}_3$	P_2O_5	K_2O	Humus
H_2O	KCl	mg kg^{-1}	$\text{mg } 100\text{gr}^{-1}$		%
8,4	7,6	11,5	18,2	31,3	1,27

Seedlings of lettuce (*Lactuca sativa*) with average weight 21.43 ± 6.41 g was purchased from the Institute of Ornamental and Medicinal Plants - Negovan, Sofia and have been planted in both greenhouses by 55 plants each in 4 rows and irrigated 3 times a week during the experiment by a local water source.

Average outdoor air temperature and relative humidity during the experimental period was 16.4 ± 7.6 °C and 60.2 ± 8.3 %, respectively according to the data from the meteo station Sofia of the National Institute of Meteorology and Hydrology.

During the experiment the following parameters were controlled and measured both in control and experimental greenhouses (lettuce groups):

1. Average level of CO_2 , indoor air temperature and relative humidity - three times a week during irrigation at noon by handset IAQ Monitor SM-2100;
2. Average level of ammonia (NH_3) - three times a week during irrigation at noon by handset Aeroqual Series 200 Monitor;
3. Average total moisture/dry matter, crude protein and ash, Ca and P content by standard laboratory methods at 1-st, 13-th, 17-th and 40-th day;
4. Vegetative biomass index (NDVI) by Greenseeker Handheld (Trimble) at 38-th day;
5. Average weight of lettuce at 1-st, 17-th and 40-th day.

The results of one factor statistical analysis are expressed as means \pm S.E.M. and were analyzed by Student t-test.

Results and Discussion

The temperature and humidity in the control greenhouse were slightly higher than those in the experimental one. During the experiment, the carbon dioxide and ammonia values in the experimental greenhouse were high, in the middle of the experiment, they kept their values, then began to decrease (Table 2). This was due to the reduction in the number of poultry reared at the end of the experiment. The average CO_2 levels in the experimental group were $602.724 \pm$

98.041 ppm and those in the control group – 461.076 ± 24.810 ppm, but in the beginning of the experiment were 747 and 440 ppm, respectively.

Table 2: Microclimate indicators during the experiment at noon

	Temperature, ° C		Humidity, %		CO ₂ , ppm		NH ₃ , ppm	
	Experimental	Control	Experimental	Control	Experimental	Control	Experimental	Control
Mean	23.87417	24.50583	60.63833	64.315	602.72417	461.0758	10.0358	0.183333
±Sx	2.834167	2.949167	3.102222	4.754167	98.040833	24.81042	6.99681	0.216111

It is noteworthy that the ammonia values in the experimental greenhouse vary. At the inlet of the duct the values were on average 3.6 ppm, and at 50 cm from it at a height of 1.5 m from the surface there is an increase to an average of 8.9 ppm. At the opposite corner of the greenhouse, the ammonia values were average: at the plant level - 1.7 ppm and at a height of 1.5 m - 1.9 ppm. These results are similar to those, reported by *Jones et al. (2013)*, who also established close dependence between ammonia concentration and outdoor air velocity. Although indoor temperature was identified as main variable influencing NH₃ emissions, other variables, such as ventilation rate and bird activity, may also be influencing those emissions (*Walker et al., 2014*).

What was seen in the greenhouse where the experiment was conducted was that in the far end of the greenhouse the plants were significantly larger and more developed, with a better shaped leaves. At the entrance to the greenhouse, where the amount of CO₂ was less, the plants were smaller, poorly developed, less leavening, i.e. there was a lag in growth, at the expense of the plants located near the northern and southern walls of the greenhouse, ie. second and first line. *Lakshmi et al. (2017)* in their experiment with sunflower, planted in a high CO₂ chamber also established that though elevated CO₂ decreased the leaf, stem and seed N per cent, elevated CO₂ increased the total nitrogen uptake per plant through increase in biomass and seed yield. According to them, the recommended dose of nutrients, the nitrogen uptake, physiological N use efficiency and grain N use efficiency was high under elevated CO₂ conditions.

As we expected in the first two weeks lettuce plants in the experimental greenhouse had better development than those in the control one. The samples taken from the experimental group of plants at 17-th day showed a thicker and denser mass of leaves "on the touch" and their average weight was higher ($87 \pm$

10.2 g). We found a waxy cover on the leaves and obviously a darker green color (Pictures 3 and 4).



Picture 3. Experimental lettuce



Picture 4. Control lettuce

The average weight of the control plants samples was lower - 70 ± 8.1 g, the difference between the two groups was not significant. At the end of the experiment, however, the weight of the lettuce in the control group was greater than that of the control (298.44 ± 65.578 and 215.54 ± 83.462 respectively), although the difference was not significant, too (Table 3). Our results do not correspond well with *Lakshmi et al. (2017)* research mentioned above, about sunflower's leaf dry wt. (g per plant) and a possible reason we think was due to the fact that CO₂ treatment of lettuce in our experimental greenhouse was not "clear", but as a part of emission from a poultry house, where ammonia, other gases (incl. VOC) and dust have to be taken into account.

Table 3: Lettuce average weight

Days	Experimental group, g		Control group, g	
	mean	± Sx	mean	± Sx
17-th (n=10)	87	10.2	70	8.1
40-th (n=24)	215.54	83.462	298.44	65.578

According to the latest data from National Nutrient Database for Standard Reference Legacy Release of United States Department of Agriculture, Agricultural Research Service (2018) about the lettuce's nutrient content, the normal level of protein is about 1.36% and crude ash - 0.62%. *Januškevičius et al. (2012)* established that the crude protein content in lettuce fresh samples from the market was $0.92 \pm 0.03\%$. Our results showed that in the end of the experiment there were $1.26 \pm 0.09\%$ and $1.63 \pm 0.11\%$ of crude protein in control and experimental groups, respectively (Table 4). The difference was significant ($p < 0.01$). In the 17-th day of the experiment crude protein levels in both groups were twice higher - $2.49 \pm 0.21\%$ and $3.21 \pm 0.25\%$, respectively ($p < 0.01$). The same differences we found about the crude ash content, but levels in the control group were almost twice higher than in the experimental one and up to 10 times higher than reported as normal above, which requires more in-depth researches.

Contents of total moisture, Ca and P in both groups were similar during the whole experiment and compared to the normal ones, too.

Table 4: Lettuce content

Groups/days	Total moisture, %		Crude protein, %		Crude ash %		Ca, %		P, %	
	mean	±Sx	mean	±Sx	mean	±Sx	mean	±Sx	mean	±Sx
Control 17-th day (n=10)	83.02	2.21	2.49	0.21	7.52	2.06	1.098	0.093	0.211	0.007
Experimental 17-th day (n=10)	87.82	3.14	3.21	0.25	3.82	1.84	0.935	0.061	0.108	0.004
Control 40-th day (n=24)	95.44	1.36	1.26	0.09	1.11	0.24	0.857	0.057	0.593	0.011
Experimental 40-th day (n=24)	95.37	1.47	1.63	0.11	0.70	0.18	0.764	0.049	0.401	0.010

We also used the Vegetative biomass index (NDVI) to assess the health and viability of plants in the end of the experiment. It confirms the fact that poorly developed plants also show a lower vegetative index, while healthy and well-developed plants show index values above 0.7-0.8. The plants in both groups had almost the same NDVI index – 0.738 ± 0.0867 and 0.74 ± 0.0891 which demonstrates a relatively good development.

Conclusions

Lettuce plants in the experimental greenhouse had significantly higher crude protein content ($p < 0.01$) during the experiment than those in control group, which can be related to the increased CO_2 content.

There are no significant differences at average weight of the plants in both groups, but they had a trend to a different growth rate during the experiment, possibly due to the mixed and variable emission from the poultry house.

The total moisture, Ca and P content and the Vegetative biomass index in both groups were similar.

Mogućnosti korišćenja ugljen-dioksida iz živinarstva u stakleničkoj proizvodnji zelene salate (*Lactuca sativa*)

Ivan Yanchev, Kamelia Kancheva

Rezime

Cilj ove studije bio je da se proceni uticaj povećanih nivoa CO_2 kao jedinjenja mešovutih emisija iz živinarskih objekata (koje se vazдушnim kanalom prevoze u staklenik) na rast zelene salate i njen sadržaj. Eksperiment je izveden na eksperimentalnoj živinarskoj farmi Instituta za stočarstvo – Kostinbrod, u maju i junu 2019. godine, u trajanju od 40 dana. Tokom eksperimenta sledeći parametri su kontrolisani i mereni u kontrolnim i eksperimentalnim platenicima (grupe salate): prosečni nivo CO_2 , temperatura vazduha i relativna vlažnost u objektu, prosečan nivo amonijaka (NH_3), prosečna ukupna vlaga/suva materija, sirovi protein i sadržaj pepela, Ca i P, vegetativni indeks biomase (Vegetative biomass index - NDVI) i prosečna masa zelene salate 1., 17. i 40 dana. Dobijeni rezultati pokazali su da biljke salate u eksperimentalnom stakleniku imaju značajno veći sadržaj sirovog proteina ($p < 0,01$) tokom eksperimenta u odnosu na kontrolne grupe, što

može biti povezano sa povećanim sadržajem CO₂. Nije bilo značajnih razlika u prosečnoj težini biljaka u obe grupe, ali su imali trend drugačijeg rasta tokom eksperimenta, verovatno zbog mešovite i promenljive emisije iz živinarskog objekta. Ukupni sadržaj vlage, Ca i P i NDVI u obe grupe su bili slični.

Ključne reči: živina, ugljen dioksid, amonijak, staklenik, zelena salata, protein

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POSTER SECTION I

EVALUATION OF THE HAEMATOLOGICAL PROFILE AND SERUM ENZYMES DURING THE TRANSITION PERIOD IN DAIRY COWS

Marinela Enculescu

Research and Development Institute for Bovine, 21, Bucuresti-Ploiesti, 077015, Balotesti, Romania
Corresponding author: Marinela Enculescu, marinelaenculescu2006@yahoo.com
Original scientific paper

Abstract: The aim of this study was to investigate the changes of haematological parameters and serum enzymes during the transition period in dairy cows. The study was carried out at the Experimental Farm of the Research and Development Institute for Bovine Balotesti, Romania. A number of 60 Romanian Black and Spotted dairy cows, clinically healthy, were screened during the transition period (last 3 weeks *pre-partum* and first 3 weeks *post-partum*). Blood samples were analyzed for haematological parameters (red blood cells count, haemoglobin concentration, hematocrit percentage, red blood cells distribution width, platelet count, mean platelets volume, platelets distribution width, total white blood cells count, lymphocytes percentage, monocytes percentage, neutrophil percentage) and serum enzymes (aspartate transaminase, alanine aminotransferase, alkaline phosphatase). A significant effect of the physiological status ($P < 0.0001$) on some haematological parameters was observed. The concentration of aspartate transaminase (AST) in serum blood was significantly ($F_{critical} = 4.58$; $F_{0.05 (4; 59)} = 2.52$; $F_{critical} > F_{0.05}$; $p = 0.001$), whereas, the concentration of alanine aminotransferase (ALT) and the concentration of alkaline phosphatase (ALP) did not show differences ($P > 0.05$) during the transition period. This study could be useful in preventing metabolic diseases of dairy cows which occur due to unbalanced feeding management of dairy cows during the transition period.

Key words: dairy cows, haematological profile, serum enzymes, transition period.

Introduction

Severe economic losses in dairy farming can result from a suboptimal transition period of the animals (*Overton and Waldron, 2004*). The transition period for dairy cows is extended period from 3 weeks pre-calving to 3 weeks post-calving

(Drackley, 1999) and is characterized by distinct metabolic, hormonal, inflammatory and oxidative stress changes (Tharwat *et al.*, 2012; Sordillo and Raphael, 2013; Van Saun, 2016; Abuelo *et al.*, 2019). Maintaining health status and productivity performances during the transition period is one of the most important aspects in dairy cows farming. All the conditions predisposing (environmental, nutritional errors) to metabolic and reproductive disorders such as subclinical and clinical ketosis, fatty liver, milk fever, displaced abomasum, endometritis, metritis, mastitis (Andrieu, 2008; Mulligan and Doherty, 2008) occurring during the transition period can be evaluated with specific laboratory tests (Stockham and Scott, 2008a). Haematology and serum biochemical parameters are an essential indicator of the animals' health status (Islam *et al.*, 2019), blood reference values are used to diagnose systemic diseases (Kumar *et al.*, 2015). Serum enzymes, aspartate transaminase (AST), alanine aminotransferase (ALT) and alkaline phosphatase (ALP) are important catabolic enzymes, which play an important role in the liver function of animals (Ghada, 2014). The aim of this study was to investigate the changes in the levels of haematological parameters and serum enzymes during the transition period in dairy cows.

Materials and Methods

Animals, diet and protocol design. A total of 60 Romanian Black Spotted dairy cows, clinically healthy, were screened for blood serum indicators during the transition period (3 weeks *pre-partum*, 1 week *pre-partum*, at calving, 1 week *post-partum* and 3 weeks *post-partum*). The cows were housed under tied stanchion barn conditions. Feeding system was differentiated by physiological status (during the *pre-partum* period the diet/head/day consisted of 7 kg alfalfa hay, 15 kg brewer's yeast and 1.5 kg concentrates; during the *post-partum* period the diet/head/day consisting of 8 kg alfalfa hay, 20 kg corn silage, 1 kg sunflowers meal, 4 kg concentrates and 80 g *Saccharomyces cerevisiae*). The dairy cows received salt and water *ad libitum*. All experimental procedures were performed in accordance with the Romanian Law no. 43/2014 and the Council Directive 2010/63/EU regarding handling and protection of animals used for scientific purposes.

Blood sampling. The analyses were carried out in the Animal Physiology and Biochemistry Laboratory of the Research and Development Institute for Bovine Balotesti, Romania. For the hematological examinations, blood samples were collected aseptically from the jugular vein of each animal, 2-4 hours after the morning feeding. The amount of blood was 1-2 ml for each sample in vacutainer tubes with disodium-ethylene diamine tetra acetic acid (EDTA/Na₂) with a concentration of 1.5 mg EDTA/Na₂ per ml of blood as an anticoagulant. After

harvesting, the samples were chilled to +4 °C. For the biochemical examination of blood serum, the samples were collected in dedicated vacutainer tubes under sterile conditions. A blood sample of approximately 9 ml was taken to perform the biochemical examination. The blood samples were kept at room temperature (approximately 22-24 °C) until the serum was expressed. Serum was separated by centrifugation at 3000 rpm for 15 min and stored in aliquots at -20 °C until further analysis.

Haematological and biochemical analysis. Haematological parameters (red blood cells count, haemoglobin concentration, hematocrit percentage, red blood cells distribution width, platelet count, mean platelets volume, platelets distribution width, total white blood cells count, lymphocytes percentage, monocytes percentage, neutrophil percentage) were determined using automated hematology analyzer Abacus Junior Vet 5 (Diatron, Hungary). The blood biochemical parameters (aspartate transaminase, alanine aminotransferase, alkaline phosphatase) were estimated by using a semiautomated biochemical analyzer StarDust MC 15 (DiaSys Diagnostics Systems GmbH, Germany) and DiaSys reagents in dedicated kits.

Statistical analysis. Statistical analyses were carried out using Minitab® Statistical Software, version 18. Descriptive statistics, means (\pm standard deviations), were performed for all parameters. One-Way ANOVA was used to test the influence of the transitional period on the blood parameters studied and the Tukey's test was applied for post hoc comparisons. The difference in the mean values between intervals was considered significant at $P < 0.05$.

Results and Discussions

The recorded mean (\pm standard deviations) values of the RBC, HGB, HCT, and RDW are presented in Table 1. A significant effect of the transition period ($P < 0.0001$) was showed for RBC ($F_{(4;59)}=43$), HGB ($F_{(4;59)}=24.67$), HTC ($F_{(4;59)}=61.05$) and RDW ($F_{(4;59)}=13.27$). The red blood cell count (RBC), increased significantly on 3 weeks *post-partum* ($7.53 \pm 1.00 \times 10^6/\mu\text{l}$) as compared to other intervals, which might be due to erythropoiesis and the slow destruction of erythrocytes during the transition period. Similar values of RBC were also reported by Nazifi and Gheisari, (2008), Brijesh *et al.*, (2017) in transition dairy cows. The HGB concentration ranged from $9.14.58 \pm 0.71$ g/dl to 10.49 ± 0.76 g/dl. A significant increase in HGB was found at calving, compared to one week *pre-partum* ($P=0.001$). After calving, HGB decreased till 1 week *post-partum* and increased to 3 weeks *post-partum* significantly ($P=0.000$). The obtained HCT percentage ranged from 27.79 ± 1.44 % to 33.50 ± 2.59 %.

Table 1. Mean values of RBC, HGB, HCT, and RDW in dairy cows during the transition period

Period/ Haematological indicators n=60	RBC, 10 ⁶ /μl	HGB, g/dl	HCT, %	RDW, %
	$\bar{X} \pm \text{sd}$	$\bar{X} \pm \text{sd}$	$\bar{X} \pm \text{sd}$	$\bar{X} \pm \text{sd}$
3 weeks <i>pre-partum</i> (1)	6.31±0.52 ^c	10.18±0.65 ^{ab}	28.51±2.83 ^b	28.94±5.37 ^a
1 week <i>pre-partum</i> (2)	6.18±0.55 ^c	9.93±0.70 ^b	27.84±3.16 ^b	28.32±5.08 ^a
Calving (3)	7.15±0.92 ^b	10.49±0.76 ^a	33.50±2.59 ^a	22.08±9.24 ^b
1 week <i>post-partum</i> (4)	6.19±0.54 ^c	9.14±0.71 ^c	27.79±1.44 ^b	28.53±5.88 ^a
3 weeks <i>post-partum</i> (5)	7.53±1.00 ^a	10.09±1.04 ^b	28.03±1.68 ^b	29.15±5.31 ^a
Reference values	5-8	9-11	32-38	-

RBC=red blood cells count, HGB=hemoglobin concentration, HCT=hematocrit percentage, RDW=red blood cells distribution width. Means that do not share a letter are significantly different.

A significant increase in HCT was found at calving, compared to the other studied periods ($P=0.000$). The HCT decreased before calving, increased at calving and returned to normal soon after parturition. Red cell distribution width (RDW) helps further classify the types of anemia (*Terzano et al., 2005*). In dairy cows means values recorded for RDW decreased significantly (22.08 ± 9.24 %) at calving and increased at 3 weeks *post-partum* (29.15 ± 5.31 %). Platelets count (thrombocytes) are anuclear cytoplasmatic fragments of megakaryocytes, and play a major role in blood clotting (*Boudreaux et al., 2011; Russell, 2010*). Platelet clumping increases with time, so platelet counts should be done as soon as possible after collection to maintain accuracy, respectively within 4 hours after venipuncture (*Warren et al., 2013*). The PLT ($F_{(4;59)}=13.19$) and MPV ($F_{(4;59)}=15.11$) levels show a significant difference ($P<0.0001$) during the transition period, whereas, the PDW did not showed differences during the transition period ($P>0.05$; $F_{(4;59)}=2.32$). In this study (Table 2), the means values recorded for PLT were situated in normal physiological limits (405.2 ± 130.2 10³/μl to 528.0 ± 87.0 10³/μl). The means values obtained for MPV were below references values indicate by literature and the means values for PDW were higher than normal physiological limits. Decreased MPV might be associated with thrombocytopenia (*Stokol, 2010*).

Table 2. Mean values of PLT, MPV and PDW in dairy cows during the transition period

Period/ Haematological indicators n=60	PLT, 10 ³ /μl	MPV, fl	PDW, %
	$\bar{X} \pm \text{sd}$	$\bar{X} \pm \text{sd}$	$\bar{X} \pm \text{sd}$
3 weeks <i>pre-partum</i> (1)	528.0±87.0 ^a	6.03±0.35 ^{bc}	31.42±2.10 ^a
1 week <i>pre-partum</i> (2)	514.9±85.1 ^a	5.89±0.44 ^b	31.01±2.24 ^a
Calving (3)	405.2±130.2 ^b	6.51±0.60 ^a	32.23±1.55 ^a
1 week <i>post-partum</i> (4)	512.9±114.4 ^a	6.17±0.42 ^b	30.90±2.21 ^a
3 weeks <i>post-partum</i> (5)	512.9±114.4 ^a	6.19±0.43 ^b	31.11±1.88 ^a
Reference values	100-800	7-13	8-16

PLT=platelet count, MPV=mean platelets volume, PDW=platelets distribution width. Means that do not share a letter are significantly different.

The WBC count ($P<0.0001$; $F_{(4;59)}=13.34$) and NE percentage ($P<0.05$; $F_{(4;59)}=2.92$) showed significant differences during the period analyzed (Table 3), opposite to what was observed for the LY percentage ($F_{(4;59)}=0.29$) and MO percentage ($F_{(4;59)}=1.76$), with no statistical differences ($P>0.05$). The count of WBC increased at calving ($9.58\pm 3.16 \cdot 10^3/\mu\text{l}$) and decreased to 3 weeks *post-partum* ($7.02\pm 0.71 \cdot 10^3/\mu\text{l}$). Lymphocytes are the most numerous cell type, but the lymphocytes proportion varies with age. Changes in blood lymphocytes number reflect changes in distribution rather than changes in production or loss. Monocytes are produced in the marrow and migrate into the tissues where they differentiate further to become macrophages. Neutrophil cells of the neutrophil line are classified by the shape of their nuclei (Terzano *et al.*, 2005).

The LY and NE percentage were situated in physiological limits, whereas, the MO percentage was higher than reference limits. Monocytosis has been observed during acute stress and is caused by hemolysis, necrosis, puerperal infections (Webb and Latimer, 2011).

Table 3. Mean values of WBC, LY, MO, and NE in dairy cows during the transition period

Period/ Haematological indicators n=60	WBC, $10^3/\mu\text{l}$	LY, %	MO, %	NE, %
	$\bar{X}\pm\text{sd}$	$\bar{X}\pm\text{sd}$	$\bar{X}\pm\text{sd}$	$\bar{X}\pm\text{sd}$
3 weeks <i>pre-partum</i> (1)	9.34 ± 2.02^a	58.68 ± 8.81^a	4.08 ± 3.29^a	28.84 ± 7.17^{ab}
1 week <i>pre-partum</i> (2)	8.70 ± 1.76^{ab}	57.80 ± 8.56^a	5.02 ± 2.98^a	27.42 ± 6.86^{ab}
Calving (3)	9.58 ± 3.16^a	59.53 ± 7.55^a	4.59 ± 3.57^a	25.53 ± 10.80^b
1 week <i>post-partum</i> (4)	8.10 ± 2.47^{bc}	59.02 ± 12.08^a	5.37 ± 2.39^a	30.32 ± 10.81^a
3 weeks <i>post-partum</i> (5)	7.02 ± 0.71^c	59.66 ± 15.05^a	4.38 ± 2.78^a	30.38 ± 10.36^a
Reference values	6.5-9.5	45-61	0-4	15-41

WBC=total white blood cells count, LY=lymphocytes percentage, MO=monocytes percentage, NE=neutrophil percentage. Means that do not share a letter are significantly different.

The obtained mean values of serum enzymes in the studied dairy cows during the transition period are summarized in Table 4. The concentration of AST showed a statistical significance ($P<0.001$; $F_{(4;59)}=4.58$) during the transition period comparative with concentration of ALT ($P>0.05$; $F_{(4;59)}=4.58$) and concentration of ALP ($P>0.05$; $F_{(4;59)}=4.58$). AST (aspartate transaminase) is localized at the mitochondrial level and it is present in greater concentrations in the muscles, heart, liver, and it increases only in the case of extensive hepatic necrosis. AST is considered as the most sensitive indicator in the diagnosis of fatty liver in cows (Lubojacka *et al.*, 2005; Stojevic *et al.*, 2005). The AST mean values of dairy cows increasing in the advanced phases of pregnancy ($39.43\pm 11.47 \text{ U/L}$) and decreasing before parturition ($32.00\pm 11.41 \text{ U/L}$). ALT (alanine aminotransferase) is present in small quantities in the liver and in various tissues, particularly in the muscles. ALT

together with AST, as an index of muscular integrity. The obtained mean values for ALT were in the normal physiological limit, lower than 60 U/L.

Table 4. Mean values of AST, ALT, and ALP in dairy cows during the transition period

Period/ Serum enzymes n=60	AST, U/L	ALT, U/L	ALP, U/L
	$\bar{X} \pm \text{sd}$	$\bar{X} \pm \text{sd}$	$\bar{X} \pm \text{sd}$
3 weeks <i>pre-partum</i> (1)	39.43±11.47 ^a	33.73±19.89 ^a	63.34±29.27 ^a
1 week <i>pre-partum</i> (2)	38.43±11.45 ^a	34.57±20.25 ^a	62.23±31.30 ^a
Calving (3)	39.65±11.27 ^a	36.62±20.02 ^a	62.17±28.04 ^a
1 week <i>post-partum</i> (4)	37.10±11.26 ^{ab}	38.90±22.32 ^a	60.28±27.61 ^a
3 weeks <i>post-partum</i> (5)	32.00±11.41 ^b	34.40±21.01 ^a	53.88±28.12 ^a
Reference values	21-24	<60	10-36

AST= aspartate transaminase, ALT=alanine aminotransferase, ALP=alkaline phosphatase. Means that do not share a letter are significantly different.

ALP (alkaline phosphatase) is an enzyme founds in the liver, bone, placenta bone and in bile (*Webber et al., 2010*). In this study, higher values of ALP in serum blood (53.88±28.12-63.34±29.27 U/L) are determined during the transition period. High blood levels of ALP may indicate disease, such as osteoporosis, hepatobiliary and fatty livers disorders (*Hanley et al., 2005; Dokovic et al., 2017*). An increase of ALP in dairy cows during early lactation is proof of speedy parathyroid activation (*Campanile et al., 1997*). Higher mean value (90.90±5.97 U/L) of ALP was recorded by *Ghada (2014)* during early lactation in dairy cows.

Conclusions

During the transition period, a significant effect on red blood cells count, haemoglobin concentration, hematocrit percentage, red blood cells distribution width, platelet count, mean platelets volume, total white blood cells count, and neutrophil percentage was observed. The concentration of aspartate transaminase in serum blood was significantly, whereas, the concentration of alanine aminotransferase and the concentration of alkaline phosphatase did not show differences during the transition period. Current results confirm that from the metabolic point of view, the transition period is the most sensible for dairy cows. The information offered by blood testing is often helpful in the diagnosis, monitoring, and prognosis of a disease. This study could be useful in preventing metabolic diseases of dairy cows which occur due to poor rearing and feeding management of the cows during the transition period. Further investigations should be conducted to evaluate how the blood parameters in transitioning cows are influenced by other factors, such as the season, environment and production levels.

Procena hematološkog profila i enzima u serumu tokom prelaznog perioda kod mlečnih krava

Marinela Enculescu

Rezime

Cilj ove studije bio je da se ispituju promene hematoloških parametara i enzima u serumu tokom prelaznog perioda kod krava u proizvodnji mleka. Studija je sprovedena na eksperimentalnoj farmi Istraživačko-razvojnog instituta za govedarstvo (Research and Development Institute for Bovine), Balotesti, Rumunija. Ukupan broj od 60 rumunskih crnih i šarenih krava, klinički zdravih, je posmatrano i analizirano tokom prelaznog perioda (poslednje 3 nedelje pre teljenja/*pre-partum* i prve 3 nedelje posle teljenja/*post-partum*). Uzorci krvi analizirani su na hematološke parametre (broj crvenih krvnih zrnaca, koncentracija hemoglobina, procenat hematokrita, širina raspodele crvenih krvnih zrnaca, broj trombocita, prosečni volumen trombocita, širina distribucije trombocita, ukupna količina belih krvnih zrnaca, procenat limfocita, i monocita, procenat neutrofila) i enzime u serumu (aspartat transaminaza, alanin aminotransferaza, alkalna fosfataza). Primećen je značajan uticaj fiziološkog statusa ($P < 0.0001$) na neke hematološke parametre. Koncentracija aspartat transaminaze (AST) u krvi u serumu bila je značajna ($F_{critical} = 4.58$; $F_{0.05 (4;59)} = 2.52$; $F_{critical} > F_{0.05}$; $p = 0.001$), a koncentracija alanin aminotransferaze (ALT) i koncentracija alkalne fosfataze (ALP) nije pokazala razlike ($P > 0.05$) tokom prelaznog perioda. Ova studija bi mogla biti korisna u sprečavanju metaboličkih bolesti krava koje nastaju usled neuravnoteženog hranjenja mlečnih krava tokom prelaznog perioda.

Ključne reči: krave u proizvodnji mleka, hematološki profil, enzimi u serumu, prelazni period

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EXTERNAL CHARACTERISTICS OF HOLSTEIN-FRIESIAN BREED ON AREA OF FEDERATION OF BOSNIA AND HERZEGOVINA

Muamer Pekmez, Admir Dokso, Muhamed Brka

University of Sarajevo, Faculty of Agriculture and Food Sciences, Institute for Animal Breeding,
Zmaja od Bosne 8, 71 000 Sarajevo, Bosnia and Herzegovina
Corresponding author: Muamer Pekmez, muamer1@hotmail.co.uk
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Abstract: The aim of the present research was to study the external features of the population of Holstein-Friesian breed in the area of the Federation of Bosnia and Herzegovina. The research was carried out on 36 cows with two or more lactations. Basic body measurements of the height of withers, the hip height, pelvis width were taken with Lythin's stick, the body length and circumference of the chest were measured with measuring tape. Average wither height was 141.34 cm, hip height 142.09 cm, pelvis width 62.54 cm, body length 164.86 cm and heart-girth circumference 205.17 cm, respectively. Data were analyzed using SAS software package, by conducting the UNIVARIATE procedure for calculating the descriptive statistics for examined individuals. Besides a series of other indicators, data about the body measurements are of extraordinary importance for cattle breeding and as such can be of particular help for achievement of breeding goals. Results of the research are showing that examined individuals do not differ from the population of Holstein-Friesian cattle in the surroundings. To obtain a more complete picture about the exterior characteristics of local populations of H-F breed, it is necessary to continue the research, including a significantly larger number of animals with the wider breeding area.

Key words: body measurements, Holstein-Friesian breed, cattle breeding.

Introduction

Since the animal production, with the special emphasis on the milk production, requires a wide array of knowledge, it is of crucial importance for improvement in milk production to have as much data on disposal as it is possible. Body measurements are of particular importance for both farm management and selection. Therefore, monitoring of body measurements is regular practice on the big farms and new technologies, especially artificial intelligence, have enabled the more precise estimation of body measures.

If we take into consideration the selection for improvement of the Holstein-Friesian breed, it is possible to conclude that validation of body measurements differs from country to county. I.e. body measurements that are related to the milk yield and dry matter intake are the most validated in Israel since the selection is mainly directed towards increased milk production. (*Schaefer et al., 1984; Cole et al., 1999; Gundelach et al., 2009*). On the other side, traits that are in correlation with functional characteristics are highly scored in selection indexes in Scandinavian countries. (*Yerex et al., 1987; Vinson et al., 1987*.)

The aim of this work is to get insight into the characteristics of the Holstein-Friesian breed of cattle in the Federation of Bosnia and Herzegovina. The examination has been conducted on the sample of 36 cows that are currently at least in the second lactation. The conclusions about the state of the body frame of Holstein-Friesian cattle will be based on statistically processed data about the wither height, hip height, pelvis width, hearth-girth circumference and body length.

Material and Methods

Data about the body measurements were collected from the sample of 36 cows of Holstein-Friesian breed placed on the farm PD Butmir. Data about the wither height, hip height, heart-girth circumference, body length and pelvis width was collected. Due to the growth dynamics, only cows that are at least in the second lactation were included in the research. Regarding the production system, it needs to be emphasized that cows are kept in tie-stall housing.

The research has been conducted on the farm PD Butmir whose tradition of animal production extends from the far 1893. when it was established as the Agricultural station Butmir. The production capacities of the farm are approximately 600 milking heads, 300 calves and 350 beef heads and heifers respectively.

Hip height was measured by using the Lydtin's stick. Namely, this body measurement is correctly determined by positioning the Lydtin's stick narrow to the

ground under the angle of 90° from the front edge of tuber coxae. The point in which hip height is determined is located at the position which represents the intersection between the line that connects front edges of tuber ischii and middle spinal line.

Wither height of animal was measured with the Lydtin's stick as well as above mentioned hip height. Namely, wither height is determined in the highest point of cow's wither with the stick positioned narrow to the ground.

Body length and hearth-girth circumference were measured with measuring tape. Body length was measured from the chest bow to the tuber ischii. Hearth-girth circumference was measured in a way that measuring tape was pulled behind the front legs of the animal.

Data were analyzed using SAS software package, by conducting the UNIVARIATE procedure for calculating the descriptive statistics for examined individuals. The mentioned procedure provides us with the specific insight in sample and enables the comparison with the results of other scientists.

Results and Discussion

Basic characteristics of Holstein-Friesian's cattle breed body frame were examined with this research. Particularly, it is a milk breed which distinguishes itself by very big body frame. Furthermore, it is necessary to take in consideration that body frame is influenced by many genetic and para-genetic factors (feeding and nutrition, production system etc.). Results of the research are given in the Table 1.

Table 1. Body measurements of Holstein-Friesian cattle breed

	Wither height	Hip height	Body length	Hearth-girth circumference	Pelvis width	Lactacion
Mean	141.34	142.09	164.86	205.17	62.54	2.74
Standard error	0.72	0.76	1.82	1.49	0.45	0.18
Median	141.00	142.00	166.00	204.00	63.00	2.00
Modus	140.00	142.00	163.00	197.00	63.00	2.00
Standard deviation	4.28	4.52	10.74	8.84	1.61	1.05
Variance	18.29	20.43	115.30	78.15	2.60	1.11
Range	21	20	52	42	5	4
Minimum	131	133	134	188	60	2
Maximum	152	153	186	230	65	6

Results from Table 1 are showing that the average wither height is within the acceptable span for Holstein-Friesian breed. Furthermore, during the observation of these results, it is necessary to take different breeding goals into consideration. Namely, traits related to fitness are more emphasized by breeding programmes, which also implies somewhat smaller body-size of the animal. On the other side, the breeding goal defined by the German Holstein breeders Association (DHV – Deutscher Holstein Verband) is to have the animals with wither height between 145 and 156 cm. Furthermore, it will be a word about the research of other authors and we will compare them with ours.

If we compare our results with those obtained by *Yerex et al. (1987)*, it is possible to notice somewhat lower wither height - 138.2 ± 2.28 cm, heart-girth circumference - 190.2 ± 1.98 cm and body length of 156.8 ± 2.13 cm. Since these results are from 1987., it is possible to notice somewhat smaller body measures than ours, which is connected with the above-mentioned selection for increased milk yield.

Similar results were confirmed by *Vinson et al. (1987)*. However, it needs to be emphasized that besides mentioned body measures, pelvis width was a little bit increased - 65.5 ± 2.9 cm. Somewhat lower standard deviations in both cases can be explained by significantly greater samples.

Our results do not differ significantly from the work provided by *Cole et al. (1999)* in which the relation of the selection of Holstein-Friesian breed on increased milk production and body size of cows was examined. It was discovered that producers prefer bigger animals with increased body size, even though line selected for lower body size shown significantly longer production life.

Schaefer et al. (1984) analyzed the impact of body measurements on problems with partus and obtained the very similar data – wither height 139.1 ± 4.23 cm, hip height 140.0 ± 3.96 cm, pelvis width 62.6 ± 2.85 cm, chest circumference 202.05 ± 9.05 cm. The results of this research shown that the selection for increased milk yield implies a higher incidence of problems during the partus since bigger animals have more problems with the pelvis. On the other hand, *Gundelach et al. (2009)* concluded that Holstein-Friesian cows with a pelvis that is narrower than 55 cm have much more problems during the partus than those with the wider pelvis.

In research conducted by *Corqueira et al. (2013)*, somewhat larger body measurements of animals were noticed – wither height 141.1 ± 4.72 cm, hip height 144.2 ± 4.47 cm, body length 170.8 ± 8.31 cm, pelvis width 55.9 ± 4.17 cm i hearth circumference 206.8 ± 10.43 cm.

Conclusions

With the insight in previous research and in-field state, it is possible to conclude that, the body size of Holstein-Friesian cows increased because of the selection on increased productivity. On the other hand, selection for increased productivity has significantly affected longevity, which has an economic impact. Therefore, fitness-related traits will be more validated within the selection indexes for desired breeding goal.

Also, it is possible to conclude that our results do not differ from the results of other authors. It will be inevitable to pay more emphasis to the research of the impact of above-mentioned traits on animal's longevity.

Furthermore, the increased body size of Holstein-Friesian cows implies even greater challenges in terms of design and construction of housing. There is also a common need for technological innovations for farmers that would contribute to the overall well being of animals.

Karakteristike telesnog okvira populacija holštajn-frizijske rase na području Federacije Bosne i Hercegovine

Muamer Pekmez, Admir Dokso, Muhamed Brka

Rezime

Cilj ovoga rada bio je utvrditi određene eksterijerne karakteristike populacija krava holštajn – frizijske rase na području Federacije Bosne i Hercegovine. Istraživanjem je obuhvaćeno 36 krava sa zaključene dve i više laktacija. Utvrđene su vrednosti visine grebena, visina krsta, širine karlice Lythinovim štapom, dužine tela i obima grudi sa mernom trakom. Prosečna vrednost visine grebena kod istraživanih krava bila je 141,34 cm, visine krsta 142,09 cm, dužine tela 164,86 cm, obima grudi 205,17 cm, širina karlice 62,54 cm. Statistička obrada podataka urađena SAS softverskim paketom, primenom UNIVARATE metode za izračunavanje deskriptivne statistike istraživanih karakteristika. Podaci o telesnim merama, pored niza drugih pokazatelja su od izuzetnog značaja za selekcijski rad te kao takvi mogu značajno pomoći u

postizanju postavljenih odgajivačkih ciljeva. Rezultati istraživanja pokazuju da ispitivane jedinke ne pokazuju značajne razlike u odnosu na populacije holštajn-frizijske rase u okruženju. Za potpuniju sliku o eksterijernim karakteristikama lokalnih populacija H-F pasmine neophodno je nastaviti istraživanje, uključujući veći broj životinja sa šireg područja istraživanja.

Cljučne riječi: telesne mere, holštajn-frizijska rasa, selekcija goveda

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THE INFLUENCE OF BULLS ON THE QUALITY OF SEMEN OF HOLSTEIN-FRIESIAN BULLS

Miloš Marinković¹, Predrag Perišić², Dušica Ostojić Andrić¹, Vlada Pantelić¹, Nikola Molerović¹, Nenad Mičić¹, Vladimir Živković¹

¹Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080, Belgrade-Zemun, Republic of Serbia

²Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080, Belgrade-Zemun, Republic of Serbia

Corresponding author: Miloš Marinković, majlo1989@gmail.com

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Abstract: The aim of this research was to investigate the influence of the bull i.e. genotype on the observed traits of semen quality and to determine whether there are significant differences between bulls in terms of semen production. The study included 9 bulls of the Holstein-Friesian breed from the Livestock Center of PKB Corporation. The effect of the bull on certain semen properties (ejaculate volume, sperm concentration, sperm motility in the native ejaculate, sperm dilution, number of doses per ejaculate, sperm motility after thawing) was analyzed and data were collected during 2014. During this period, a total of 326 ejaculates from 9 bulls were collected and average values for the tested properties were determined and the effect of the bull on those properties was analyzed. The results showed that there was a very significant effect of the bull ($p < 0.001$) on the degree of sperm dilution and the number of doses per ejaculate. Significant influence ($p < 0.01$) was found in ejaculate volume traits, sperm concentration, sperm motility in the native ejaculate, while in sperm motility after thawing no significant effect ($p > 0.05$) of the bull was determined. The LSD test (Least Significant Difference) was used to compare the bulls and the results of this test showed in most cases significant differences between the bulls.

Keywords: bulls, LSD test, sperm, ejaculate, Holstein-Friesian breed

Introduction

Fertility is one of the most important traits in cattle production. The use of artificial insemination has allowed for a much faster genetic progression within the cattle population, since thousands of doses can be made from one bull during the year. Therefore, it is very important to accurately evaluate the potential production of bull semen and, on this basis, to direct selection to bulls with desirable traits.

The quality of the semen significantly affects the fertility of cows because with the poor quality of the semen, more insemination is required for fertilization/conception, thus extending the service period and the calving interval. The production and quality of bull semen is strongly influenced by genetic factors such as breed and individual traits. In addition, sperm production and quality are conditioned by other factors, such as diet, bull housing and care, body size and body weight, age, environment conditions, ejaculation frequency and sperm acquiring, preservation and storage skills. Of all the factors that influence the quality of bull semen in this paper, special emphasis was placed on the influence of the bull i.e. bull genotype on the tested traits.

Mathevon et al. (1998) have established a heritability coefficient for ejaculate volume, sperm concentration, sperm motility, sperm count, sperm count per ejaculate with good motility, of 0.24, 0.52, 0.31, 0.38, and 0.49, respectively, in young bulls and 0.44, 0.36, 0.01, 0.54 and 0.64, respectively, for adult mature bulls. The same authors have also established a coefficient of repeatability for semen quality traits that ranged from 0.41 to 0.64. *Mathevon et al. (1998)* have analyzed, among other things, the effect of the bull on the investigated properties and their results indicate a significant influence ($p < 0.001$) of the bull on ejaculate volume, sperm concentration and motility, total sperm count and total number of motile sperm.

Asad et al. (2004) find a significant effect ($p < 0.001$) of genotype on ejaculate volume, sperm concentration and motility. The genetic variability of semen quality traits has been confirmed by many authors (*Raju et al. 1982; Rao and Rao 1978; Hafez 1993*).

Estimated heritabilities for ejaculate volume, progressive sperm motility, sperm concentration, sperm count in the ejaculate and number of live sperm were 0.15, 0.12, 0.22, 0.16 and 0.12, respectively, and genetic correlations between these five traits ranged from 0.02 to 0.99 (*Yin et al., 2019*). *Berry et al. (2019)* have found in their studies that the heritability coefficient for semen quality traits ranges from 0.13 - 0.34 and the repeatability coefficient from 0.22 to 0.45. The results of this work emphasize the great genetic variability of semen quality traits, some of which are highly heritable, so that proper selection should lead to improved semen production. Determination of the coefficient of repeatability for various traits of semen quality allows us to predict the future production potential of young bulls. In this way, it starts with selecting the animal of the preferable traits early on. Assessment of heritability indicates to what extent the traits examined will be passed on from generation to generation. Therefore, the genotype, that is, the hereditary basis of the individual, together with the environmental factors and conditions of care and housing, significantly affect the production of sperm in bulls of the Holstein-Friesian breed.

Material and Methods

Research to produce this work was carried out at the Livestock Center of PKB Corporation in Belgrade. During the data collection period, 12 bulls of the Holstein-Frisian breed were used in the artificial insemination program, and the study included the results of 9 bulls. Data refer to year 2014. The entire process of producing semen doses at the Center was followed, starting with the ejaculation of bulls and ending with the control of semen quality after thawing.

Most bulls ejaculates are take twice a week, usually on Mondays and Fridays. The ejaculate is taken from a bull once a day, while it is rare that bulls are used twice for jumping. Collection of ejaculates is performed by an artificial vagina while another bull is used to cause a sexual reflex or jump.

A general examination of sperm involves an assessment of color, odor and consistency. Ejaculates that are dirty or with blood/pus have to be discarded but noted in records for the current year. If the sperm passes the general examination, the volume of ejaculate, density and motility of the sperm are evaluated. The ejaculate volume is determined volumetrically in a graduated sperm collector. Ejaculate density (sperm count in 1 ml of ejaculate) is measured using a photometer while motility is estimated based on the number of sperm exhibiting progressive motility. Observation is performed using a microscope (magnification 20 - 40x) and ratings are given for motility from 1 to 5.

The ejaculate that meets the basic criteria is then diluted and divided into a number of doses, which are packed in form of paillettes. The most common is the mean degree of dilution (1:10 - 1:15) and the doses obtained have up to 20 million spermatozooids. The most commonly used diluent is AndroMed, and the diluted semen are vacuum packed into 0.22mL straws (paillettes) and then sealed with ultrasound. Subsequently, the usual procedure of deep freezing semen doses is applied. The first control of deep-frozen semen is carried out 24 hours after freezing, when the percentage of progressively moving spermatozoa is determined, which should be at least 50%.

The semen quality traits analyzed were: 1) ejaculate volume (in mL, 2) sperm concentration (in $10^6/\text{mL}$., 3) sperm motility in native ejaculate (grade 1 to 5), 4) degree of sperm dilution (sperm: thinner ratio), 5) number of doses per ejaculate, 6) motility of sperm after thawing (%). For these properties, the basic parameters of the descriptive statistics (average, minimum, maximum, standard deviation) were calculated. The method of analysis of variance (F test) was used to examine the influence of bulls i.e. genotype on semen quality, and the significance of differences of average values of observed traits between individual levels of observation were tested using the Least Significant Difference Test (LSD - test). The number of ejaculates whose quality parameters were analyzed was not the

same for all traits tested. The minimum values that the semen must satisfy in order to be used for artificial insemination are: ejaculate volume - 2mL, sperm concentration - $800 \times 10^6/\text{mL}$, motility score - 4- (75-80% progressively motile sperm), motility after thawing - 50 %. This decrease in the total number of ejaculates, which are finally taken for analysis, is about 50% of the total number of ejaculates collected, and there are large differences between the bulls. Statistical processing of the obtained data was performed with the software package "STATISTICA 6.0 StatSoft, 2001".

Results and Discussion

Table 1 lists the trait values of nine bulls in 2014. These data show that the average volume of the ejaculate was 5.1 mL and that the lowest volume was 1 and the highest 14 mL. The average sperm concentration was $1067.8 \times 10^6/\text{mL}$, the lowest was 30 and the highest was $2600 \times 10^6/\text{mL}$. Data obtained by *Miljkovic (1995)* show the average sperm concentration of $1200 \times 10^6/\text{mL}$ which is higher than the values obtained by analyzing the examined quality traits of bull semen in 2014. The sperm motility scores in the native ejaculate ranged from 1 to maximal 5, but the mean score was 3.4. The sperm dilution rate averaged 16.3 but the variation in this property was remarkable. The lowest dilution rate was only 9 and the highest was 27. As a result, the number of doses varied from 95 to 920, while the average was 368.8 doses per ejaculate. The average sperm motility after thawing was 51%, the minimum was 10 and the maximum was 75%. It should be noted that ejaculates that are dirty or with admixtures of blood and pus were immediately discarded and not used for analysis, but such ejaculates have been recorded and are attached.

Table 1. Parameters of descriptive statistics for the examined traits of bull semen quality in 2014

Trait	N	Average	Minimum	Maximum	Std. Dev.
Ejaculate volume, mL	326	5.1	1.0	14.0	1.95
Concentration ($10^6/\text{mL}$)	326	1067.8	30.0	2600.0	490.73
Mobility, evaluation	326	3.4	1.0	5.0	0.87
Dilution rate (Sperm: Thinner)	158	16.3	9.0	27.0	3.44
Number of doses from single ejaculate	157	368.8	95.0	920.0	145.60
Sperm motility after thawing (%)	153	51.0	10.0	75.0	11.01

Duret et al. (2008) have analyzed the properties of Holstein breed bull semen and found, among other things, an average ejaculate volume of 4.07 mL, a spermatozoid concentration of $1.08 \times 10^9/\text{mL}$, and sperm motility of 55.89%. Similar

values were obtained by *Ghasemi and Ghorbani (2014)*, they have compared three genotypes for the FSH β gene and obtained values for ejaculate volume from 3.1 to 4.7 mL; for sperm concentration from $967.8 \times 10^6/\text{mL}$ to $1151.3 \times 10^6/\text{mL}$ and for sperm motility after thawing, values from 58.96% to 60.92%.

By analyzing the collected data on the examined properties of bull ejaculates and comparing them with the results of other authors, it can be concluded that the averages of the traits found in Table 1 satisfy the basic criteria of ejaculate quality.

Table 2. Average values and average errors for bull semen quality traits examined in 2014

Bull	Ejaculate volume, Average	Ejaculate volume, Std. error	Concentration ($10^6/\text{mL}$) Average	Concentration ($10^6/\text{mL}$) Std. error	Motility Average	Motility Std. error	N
1	4.4	0.238	795.8	61.701	2.92	0.112	40
2	6.5	0.289	1297.4	75.100	3.85	0.136	27
3	5.9	0.250	1187.9	65.039	3.50	0.118	36
4	4.5	0.229	720.0	59.510	2.84	0.108	43
5	3.1	0.229	1520.0	59.510	3.86	0.108	43
7	6.3	0.284	682.9	73.747	2.42	0.134	28
8	5.2	0.232	1126.2	60.214	3.67	0.109	42
9	4.4	0.229	897.2	59.510	3.16	0.108	43
10	7.9	0.307	1548.7	79.656	4.21	0.145	24
F exp.	28.889**		24.619**		21.219**		326

***($p < 0.001$) **($p < 0.01$) *($p < 0.05$) n.z. ($p > 0.05$)

Table 3. Average values and average errors for bull semen quality traits examined in 2014

Bull	Dilution rate, Average	Dilution rate Std. error	N	Number of doses Average	Number of doses Std. error	N	Sperm motility after thawing Average (%)	Sperm motility after thawing Std. error (%)	N
1	13.7	0.816	12	312.7	35.467	11	47.8	3.590	9
2	16.2	0.632	20	444.2	26.303	20	53.5	2.408	20
3	17.3	0.666	18	415.3	27.726	18	44.7	2.539	18
4	15.8	1.413	4	398.8	58.815	4	42.5	5.385	4
5	19.6	0.508	31	273.6	21.127	31	51.3	1.934	31
7	12.0	1.264	5	334.0	52.606	5	54.0	4.817	5
8	14.7	0.534	28	303.8	22.230	28	50.0	2.112	26
9	14.8	0.686	17	326.8	28.529	17	54.1	2.612	17
10	16.9	0.589	23	534.8	24.528	23	54.3	2.246	23
F exp.	10.404***		158	11.3774***		157	1.846 ^{n.z.}		153

Tables 2 and 3 show the average values and standard errors of the average for the tested semen quality traits for each bull separately. Table 4 shows the results of the LSD test, out of 36 cases of bull comparisons, those with significant differences were identified ($p < 0.001$). Only the most stringent level of significance was used ($p < 0.001$).

The average for ejaculate volume was the highest for bull number 10 and it was 7.9 mL, while the average was the lowest in bull number 5 and it was 3.1 mL (Table 2). The analysis showed that the bull had a significant effect on ejaculate volume. As the significant influence of the bulls was determined, in the following analysis, using the LSD test, a comparison was made between the bulls to determine the significance of the difference between them (Table 4). The results of that test found that in 11 of the 36 cases of comparison, there was no significant difference between the bulls, but in the remaining 25 cases of comparison, a very significant difference ($p < 0.001$) between the bulls was established. These results particularly highlight bulls 10 and 5, which showed a very high difference compared to all other bulls.

The average values for sperm concentration showed the highest concentration of $1548 \times 10^6/\text{mL}$ in bull 10, while the lowest average concentration of $683 \times 10^6/\text{mL}$ was established in bull 7 (Table 2). It is interesting that in case of the bull 5, which had the lowest average ejaculate volume, almost the best average concentration ($1520 \times 10^6/\text{mL}$) was established. Using the F test, it was found that the bull significantly affected the concentration of spermatozooids in the ejaculate. The bulls were then compared with each other using the LSD test to determine the significance of the difference between them (Table 4). The results of this test showed that in 14 out of 36 cases of comparison, there was no significant difference between the bulls, but in the remaining 22 cases of comparison, a very significant difference ($p < 0.001$) was found between the bulls.

Average scores for sperm motility in the native ejaculate showed that the bull 10 had the best average score of 4.21 and the bull 7 had the worst average score of 2.42 (Table 2). This trait has been found to be under a significant effect of the bull. Therefore, an LSD test was also performed to determine the significance of the difference between the bulls (Table 4). Applying this test, it was found that in 16 out of 36 cases of comparison, there was no significant difference between the bulls but in the remaining 20 cases of comparison, there was a very high difference between them ($p < 0.001$).

The next three studied properties (dilution rate, number of doses, and motility after thawing) resulted in a decrease in the total number of ejaculates used to calculate their average values, which are presented in this paper (Table 3). This is because ejaculates that lack the proper sperm concentration and motility score are already rejected. This decrease was about 50% of the total number of ejaculates, with

differences between the bulls. The worst in this regard were the bulls 4 (4/43) and 7 (5/28) and the best was the bull 10 (23/24) which had only one ejaculate that was not prepared for freezing.

The degree of dilution is a property that is highly dependent on sperm concentration and motility score. The results in Table 3 show the best average dilution rate for bull 5 of 19.6. Such a high average dilution rate is explained by the fact that this bull's ejaculates were characterized by a very good sperm concentration and motility score, but at the same time by a low average ejaculate volume, which required such dilution. The lowest average degree of dilution was recorded for bull number 7 - 12. Analysis of this property, using the F test, revealed a significant influence of the bull on its expression. Further analysis involves conducting an LSD test to determine the significance of differences between bulls (Table 4). The results of this test showed that in 27 of the 36 cases of mutual comparison there was no significant difference between the bulls while in the remaining 9 cases of the mutual comparisons there was a significant difference between the bulls. Particularly prominent was the bull 5, which showed a very high difference ($p < 0.001$) compared to most of the other bulls.

Table 4. Effect of bull on ejaculate volume, sperm concentration, sperm motility in native ejaculate, sperm dilution and number of doses per ejaculate (LSD test)

Bull	Ejaculate volume	Sperm concentration	Sperm motility in native ejaculate	Sperm dilution	Number of doses
1	(2,3,5,10)***	(2,3,5,8,10)***	(2,3,5,8,10)***	(3,5)***	10***
2	(1,4,5,8,9,10)***	(1,4,7,9)***	(1,4,7,9)***	5***	(5,8)***
3	(1,4,5,9,10)***	(1,4,5,7,10)***	(1,4,7,10)***	(1,7)***	5***
4	(2,3,5,7,10)***	(2,3,5,8,10)***	(2,3,5,8,10)***	/	/
5	(1,2,3,4,7,8,9,10)***	(1,3,4,7,8,9)***	(1,4,7,9)***	(1,2,7,8,9,10)***	(2,3,10)***
7	(1,4,5,9,10)***	(2,3,5,8,10)***	(3,5,8,9,10)***	(3,5,10)***	10***
8	(2,5,10)***	(1,4,5,7,10)***	(1,4,7)***	5***	(2,10)**
9	(2,3,5,7,10)***	(2,3,10)***	(2,5,7,10)***	5***	10***
10	(1,2,3,4,5,7,8,9)***	(1,3,4,7,8,9)***	(1,3,4,7,9)***	(5,7)***	(1,5,7,8,9)***

***($p < 0.001$)

Analysis of the effect of the bull on the number of doses per one ejaculate showed that there was a significant effect on this trait. Since the significant influence of the bull on the number of doses has been confirmed, an LSD test followed to examine the significance of the differences between the bulls (Table 4). Based on this test, it

can be concluded that the bull 10 differed significantly from all other bulls. These differences were generally very highly significant ($p < 0.001$) and this only confirmed the results in Table 3 where it is evident that this bull had by far the best average number of doses per ejaculate. However, if we exclude the bull 10 and pay attention only to the remaining bulls, the results show that in most cases there were no significant differences between them.

Sperm motility after thawing is the last and most important property tested. The values of this trait determine whether doses of one ejaculate will be used for insemination. The average values for this trait in these nine bulls ranged from 42.5 to 54.3%, but sperm motility after thawing is the only trait found that was not under significant effect of the bull.

It can be concluded that the bull significantly affected the volume of the ejaculate, sperm concentration, sperm motility in the native ejaculate, the degree of dilution and the number of doses per ejaculate, and had no significant effect on the sperm motility after thawing. These are the expected results as the motility of sperm after thawing is more influenced by the freezing and thawing process.

Conclusion

Based on the results of research conducted at the Livestock Center of PKB Corporation on the impact of various factors on the production and quality of bull sperm, the following conclusions can be drawn:

- analysis of the data showed that the ejaculates on average met the basic criteria with less or more variation of traits,
- the influence of the bull on the tested traits – it can be concluded that the bull significantly affects the volume of the ejaculate, the concentration of sperm, the motility of the sperm in the native ejaculate, the degree of dilution and the number of doses per ejaculate, and has no significant effect on the motility of the sperm after thawing.
- some semen quality traits are medium to high in regard to heritability, and appropriate selection on these traits could lead to progress in semen production.

Uticaj bika na kvalitet semena bikova holštajn-frizijske rase

Miloš Marinković, Predrag Perišić, Dušica Ostojić Andrić, Vlada Pantelić, Nikola Molerović, Nenad Mičić, Vladimir Živković

Rezime

Cilj ovog istraživanja je da se ispita uticaj bika odnosno genotipa na posmatrane osobine kvaliteta semena i da se ustanovi da li postoje značajne razlike između bikova u pogledu proizvodnje semena. Istraživanjem je obuhvaćeno 9 bikova holštajn-frizijske rase iz Centra za stočarstvo PKB Korporacije. Analiziran je uticaj bika na određena svojstva semena (zapremina ejakulata, koncentracija spermatozoida, pokretljivost spermatozoida u nativnom ejakulatu, razrjeđenje sperme, broj doza od jednog ejakulata, pokretljivost spermatozoida posle odmrzavanja) i podaci su prikupljeni tokom 2014 godine. U ovom periodu sakupljeno je ukupno 326 ejakulata od 9 bikova i utvrđene su prosečne vrednosti za ispitivana svojstva i analiziran je uticaj bika na ta svojstva. Rezultati su pokazali da postoji veoma značajan uticaj bika ($p < 0.001$) na stepen razrjeđenja sperme i broj doza od jednog ejakulata. Značajan uticaj ($p < 0.01$) je utvrđen kod osobina zapremine ejakulata, koncentracija spermatozoida, pokretljivost spermatozoida u nativnom ejakulatu dok je kod pokretljivosti spermatozoida posle odmrzavanja utvrđeno da ne postoji značajan uticaj ($p > 0.05$) bika. LSD test (*Least Significant Difference*) je korišćen za međusobno poređenje bikova i rezultati ovog testa su pokazali u većini slučajeva značajne razlike između bikova.

Ključne reči: bikovi, LSD test, spermatozoidi, ejakulat, holštajn-frizijska rasa

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THE EFFECT OF PARTICULAR PARAGENETIC FACTORS ON FERTILITY AND MILK PERFORMANCE PROPERTIES OF COWS

Ivan Ćosić, Dragana Ružić-Muslić, Nevena Maksimović, Bogdan Cekić, Dragan Nikšić, Nenad Mičić, Miloš Marinković

Institute for Animal Husbandry, Autoput 16, P. Box 23, 11080, Belgrade-Zemun, Republic of Serbia
Corresponding author: Ivan Ćosić, ivancosic58@yahoo.com
Original scientific paper

Abstract: The main goal of breeding and selection work in dairy cattle farming is to select animals with genetic predispositions for high quality milk production, which can exhibit their potential under adequate holding conditions and are superior to previous generations in their production performances. A number of factors, of varying duration and intensity, can have impact on the phenotypic expression of milk performance traits. Fertility traits are gaining increasing importance in most of the approved programs for the genetic improvement of dairy cattle, as they have increasing impact on milk production. Long lasting unilateral selection aimed only at improvement in milk properties had a negative impact on the reproductive performance. The analysis of the impact of reproductive and milk performance parameters of Holstein-Friesian cows included a total of 364 cows reared on 4 farms of the Agricultural Corporation of Belgrade (PKB). The calving occurred during four seasons (spring, summer, autumn and winter) and during a three year period (2013-2015). The analysis included the following fertility traits: first service period (SP1), subsequent service period (SP2) and calving interval (CI), while specific observed milk performance traits included were: milk yield - first (MY1) and milk yield - subsequent (MY2), also milk fat content - first (MFC1) and milk fat content - subsequent (MFC2), and lactation duration – first (DL1) and subsequent (DL2). The studied milk performance traits had higher average values in the second than in the first lactation, the same trend was observed in fertility traits.

Keywords: Holstein-Friesian breed, reproductive performance, milk performance traits, cows

Introduction

The main goal of breeding and selection work in dairy cattle farming is to select animals with genetic predispositions for high quality milk production, which can meet their potential under adequate holding conditions, supercede previous generations in production performance and pass on the performance potential to new generations. This is significant not only from the point of view of selection success but also economically, as stability in milk production is achieved in the long run. The application of new scientific advances and the development of breeding evaluation methods have led to significant advances in improvement of breeding cattle intended for high yielding and high quality milk production. The turnover and exchange of genetic material between countries has, in particular, influenced the need for faster and more accurate estimates of breeding value.

Material and Methods

In this paper, a total of 364 Holstein-Friesian cows bred on 4 farms of the Agricultural Corporation of Belgrade (PKB) were included in the analysis of the impact of reproductive and milk performance parameters of Holstein-Friesian cows. They calved through four seasons (spring, summer, autumn and winter) in three year period (2013-2015).

The analysis included the following fertility traits: first service period (SP1), subsequent service period (SP2) and calving interval (CI), while specific observed milk performance traits included were: milk yield - first (MY1) and milk yield - subsequent (MY2), also milk fat content - first (MFC1) and milk fat content - subsequent (MFC2), and lactation duration – first (DL1) and duration of subsequent lactation (DL2).

Phenotypic parameters, i.e., the average values of the examined milk performance and fertility traits and their correlation, were calculated using standard statistical procedures in Statistica 13.2 software (*StatSoft, 2017*).

The strength of the correlation of traits was discussed on the basis of the Roemer – Orphal classification (*Latinovic, 1996*).

Results and Discussion

According to the results of the descriptive statistics presented in Table 1, with a total of 362 cows included as a sample of the main population of the Holstein-Friesian population, the following data were obtained on the general average, standard deviation, minimum and maximum values of traits.

Table 1. Descriptive parameters for examined milk performance and fertility traits

	MY1 (kg)	MFC1 (kg)	DL1 (days)	SP1 (days)	MY2 (kg)	MFC2 (kg)	DL2 (days)	SP2 (days)	CI (days)
\bar{x}	7758.6	326.9	353.0	135.4	8077.1	333.5	348.6	140.9	413.6
N	364	364	364	364	364	364	364	364	364
Sd	1302.9	20.6	54.7	56.3	1738.5	14.6	63.3	65.5	56.1
Min	2628.0	260.0	217.0	25	2629.0	279.0	219.0	33.0	307.0
Max	11117.0	397.0	497.0	309.0	12560.0	392.0	497.0	388.0	595.0

The milk performance traits studied had higher average values in the second lactation than in the first, as well as the fertility traits. The calving interval ranged from a minimum of 307 to a maximum of 595 days between the two calves. Through a review of the literature, *Milić et al.*, (1994) conclude that a tied system, such as in the case of this study, can prolong the duration of some reproductive traits.

Table 2. Descriptive parameters for studied milk performance traits by farms

FARM	Parameter	MY1 (kg)	MFC1 (kg)	MY2(kg)	MFC2 (kg)
1	\bar{x}	7873.1	332.9	8620.2	337.8
	n	105	105	105	105
	Sd	1437.7	19.6	1753.4	21.1
	Min	4280.0	284.0	3975.0	279.0
	Max	11117.0	396.0	12080.0	392.0
2	\bar{x}	8333.5	343.5	8356.5	345.0
	n	2	2	2	2
	Sd	1021.8	2.1	1099.6	17.0
	Min	7611.0	342.0	7579.0	333.0
	Max	9056.0	345.0	9134.0	357.0
3	\bar{x}	7778.0	321.8	7865.5	330.1
	n	247	247	247	247
	Sd	1170.0	17.5	1670.8	7.8
	Min	3684.0	260.0	2629.0	281.0
	Max	10454.0	369.0	12560.0	347.0
4	\bar{x}	6647.5	373.9	7693.3	362.3
	n	10.0	10.0	10.0	10.0
	Sd	1584.4	6.8	2023.4	7.3
	Min	3251.0	360.0	3042.0	353.0
	Max	8474.0	384.0	9967.0	373.0

Table 2 describes the milk yields per farm, i.e., by farm effect as a factor, where the highest average milk yield in the first lactation was recorded in dairy cattle on farm 2 (8333.5 kg) and the lowest on farm 4 (6647, 5 kg). In the second

lactation, the highest average milk yield was recorded in dairy cows on farm head (8620 kg) and the lowest on farm 4 (7693.3 kg). The highest average milk fat yield in the first lactation was recorded in cows on farm 4 (373.9 kg) and the lowest on farm 3 (373.9 kg). According to the results in the second lactation, the highest average milk fat yield was recorded on farm 4 (362.3 kg) and the lowest on farm 3 (330.1 kg). The farm 3 had the highest number of cows (247 heads), while farm 2 (2 heads) has the least number of cows. Farm impact is certainly one of the most significant factors contributing to the phenotypic variability of milk production traits (*Trivunović, 2006*).

Table 3. Descriptive parameters for studied fertility traits by farms

FARM	Parameter	DL1 (days)	SP1 (days)	DL2 (days)	SP2 (days)	CI (days)
1	\bar{x}	357.1	141.3	358.2	147.0	419.4
	n	105	105	105	105	105
	Sd	57.8	59.8	64.9	65.8	59.4
	Min	249.0	33.0	250.0	36.0	308.0
	Max	497.0	286.0	497.0	281.0	565.0
2	\bar{x}	360.0	150.0	361.5	146.0	431.5
	n	2	2	2	2	2
	Sd	62.2	52.3	119.5	120.2	44.5
	Min	316.0	113.0	277.0	61.0	400.0
	Max	404.0	187.0	446.0	231.0	463.0
3	\bar{x}	352.0	133.5	344.6	138.4	411.9
	n	247	247	247	247	247
	Sd	53.8	55.4	62.2	65.6	55.2
	Min	217.0	25	219.0	33.0	307.0
	Max	497.0	309.0	495.0	388.0	595.0
4	\bar{x}	338.3	120.5	331.6	129.9	396.2
	n	10.0	10.0	10.0	10.0	10.0
	Sd	49.0	42.6	54.6	54.8	43.0
	Min	267.0	65.0	249.0	59.0	343.0
	Max	431.0	189.0	416.0	230.0	464.0

All data obtained on the farm 2 should be taken with reserve since the farm had the least number of cows examined. The highest number of cows was on the farm 3, where the analysis included 247 heads from a total of 364. The average minimum duration of the first service period was recorded on the farm 4, which also lead to an average shortest calving interval. A universal indicator of reproductive efficiency in cattle is the calving interval as the period between two calves. The calving interval should be 355 to 365 days and it consists of a gestation period and a service period (www.butobu.rs).

Table 4. Descriptive parameters for studied milk performance traits by seasons

SEASON	Parameter	MY1 (kg)	MFC1 (kg)	MY2 (kg)	MFC2 (kg)
1	\bar{x}	8242.1	328.4	8419.6	329.7
	N	76	76	92	92
	Sd	1232.7	15.0	1684.4	12.9
	Min	5165.0	260.0	2629.0	279.0
	Max	10531.0	366.0	12560.0	362.0
2	\bar{x}	7697.8	336.3	7710.6	337.6
	N	75	75	51	51
	Sd	1220.8	17.4	1856.4	16.0
	Min	4280.0	284.0	3042.0	313.0
	Max	11117.0	386.0	11323.0	387.0
3	\bar{x}	7370.2	321.4	7825.6	337.9
	N	101	101	111	111
	Sd	1280.4	23.3	1750.7	13.2
	Min	2628.0	273.0	4146.0	312.0
	Max	10834.0	397.0	11799.0	392.0
4	\bar{x}	7821.4	324.5	8214.5	330.2
	N	112	112	110	110
	Sd	1323.1	21.2	1666.5	14.9
	Min	3251.0	268.0	2790.0	281.0
	Max	10022.0	384.0	12080.0	377.0

Considering the effect of the season as a factor, the highest average milk yield in the first and second lactations is in the spring season, and the lowest in the autumn in the case of the first lactation, and summer in the second lactation. This can be described, and also attributed to the natural seasonality of cattle, that the largest number of cows calve in early spring, and therefore produce the highest amount of milk in the first 100 days after calving. This is a case when the actual results obtained may be poor indicators of breeding value, because a large number of environmental factors have masked effects on production performance of the animal (*Stanojević et al. 2013; Petrović D.M. et al. 2006; Đedović et al. 2012; Trifunović et al., 2002*). The maximum milk yield in the first lactation was in season 2, amounting to 11117 kg, and the minimum milk yield in the first lactation was in season 3, amounting to 2628 kg.

Table 5. Descriptive parameters for studied fertility traits by season

SEASON	Parameter	DL1 (days)	SP1 (days)	DL2 (days)	SP2 (days)	CI (days)
1	\bar{x}	348.1	129.5	350.5	140.0	421.7
	N	76	76	92	92	92
	Sd	62.6	64.5	70.9	73.7	57.6
	Min	245.0	29.0	231.0	36.0	308.0
	Max	495.0	278.0	488.0	288.0	560.0
2	\bar{x}	362.5	145.9	365.7	163.8	420.8
	N	75	75	51	51	51
	Sd	56.1	57.2	68.1	63.5	61.3
	Min	249.0	33.0	224.0	33.0	326.0
	Max	497.0	286.0	492.0	292.0	595.0
3	\bar{x}	355.1	138.7	341.4	131.5	417.2
	N	101	101	111	111	111
	Sd	52.5	53.2	51.9	53.6	58.4
	Min	233.0	52.0	219.0	39.0	326.0
	Max	474.0	282.0	477.0	296.0	562.0
4	\bar{x}	348.1	129.3	346.4	140.5	399.7
	N	112	112	110	110	110
	Sd	49.7	51.8	64.1	68.3	47.3
	Min	217.0	0.0	225.0	40.0	307.0
	Max	497.0	309.0	497.0	388.0	565.0

The shortest average duration of the first service period was recorded in animals which calved in season 4, and of the second service period in season 3, and the average longest duration of the service period was recorded in season 2. Studies have shown that calving season as an observed factor of the fertility of cows that have calved in the fall and / or winter has a bearing on fertility, and such cows have the least reproductive problems. They have the shortest insemination interval as well as the shortest service period. The largest number of calvings occurred in the autumn and winter season, which corresponds to the maximum production of cows that they achieve during the early spring. *Weigel (2004)* considers that the greatest challenge today is to achieve an optimal service period for the highest yielding dairy cows.

Table 6. Descriptive parameters for studied milk performance traits by years

YEAR	Parameter	MY1 (kg)	MFC1 (kg)	MY2 (kg)	MFC2 (kg)
2013	\bar{x}	7982.7	324.5	8170.2	323.3
	N	115	115	92	92
	Sd	1358.7	25.9	1856.1	14.8
	Min	2628.0	260.0	2629.0	279.0
	Max	10454.0	397.0	12560.0	373.0
2014	\bar{x}	7649.6	326.0	8120.5	336.1
	N	214	214	219	219
	Sd	1280.1	17.3	1687.5	12.5
	Min	3684.0	268.0	2790.0	299.0
	Max	11117.0	379.0	11991.0	387.0
2015	\bar{x}	7688.7	340.4	7736.5	340.5
	N	35	35	53	53
	Sd	1191.2	14.0	1728.9	13.4
	Min	4587.0	324.0	3418.0	326.0
	Max	9870.0	386.0	11667.0	392.0

The cows which calved in 2014 achieved the best average milk yield in the second lactation of 8120.5 kg. While the average yield of milk fat was the highest in 2015 in the second lactation and the lowest in 2013.

Table 7. Descriptive parameters for studied fertility traits by age

Year	Parameter	DL1 (days)	SP1 (days)	DL2 (days)	SP2 (days)	CI (days)
2014	\bar{x}	346.5	126.1	339.3	133.6	385.7
	N	115	115	92	92	92
	Sd	50.3	51.2	63.6	68.8	41.3
	Min	245.0	25	225.0	36.0	307.0
	Max	497.0	284.0	497.0	388.0	521.0
2015	\bar{x}	354.4	138.3	350.9	140.3	416.3
	N	214	214	219	219	219
	Sd	56.1	57.8	61.5	62.3	55.0
	Min	217.0	33.0	219.0	33.0	308.0
	Max	497.0	309.0	492.0	302.0	565.0
2016	\bar{x}	365.9	148.0	355.5	155.8	450.9
	N	35	35	53	53	53
	Sd	58.6	60.3	69.7	71.2	58.6
	Min	276.0	60.0	224.0	42.0	339.0
	Max	495.0	278.0	492.0	296.0	595.0

The average longest duration of first and second lactation was calculated in 2016. The minimum average duration of the service period is recorded in 2014, resulting in the shortest calving interval. The service period is the interval from calving to subsequent fertilization and is one of the most important fertility traits.

Table 8. Phenotypic correlations of observed trait

	MY1 (kg)	MFC1(kg)	DL1(day)	SP1(day)	MY2(kg)	MFC2(kg)	DL2(dan)	SP2(day)	CI(day)
MY1 (kg)	1								
MFC1 (kg)	-0.251**	1							
DL1 (day)	0.196**	-0.047 ns	1						
SP1 (day)	0.130*	-0.053 ns	0.947**	1					
MY2 (kg)	0.410**	-0.006 ns	0.123*	0.075 ns	1				
MFC2 (kg)	-0.272**	0.279**	0.136**	0.155**	-0.195**	1			
DL2 (day)	0.025 ns	0.056 ns	0.053 ns	0.035 ns	0.162**	0.055 ns	1		
SP2 (day)	0.006 ns	0.044 ns	0.046 ns	0.048 ns	0.071 ns	0.043 ns	0.879**	1	
CI (day)	0.147**	-0.064 ns	0.966**	0.981**	0.099 ns	0.149**	0.050 ns	0.059 ns	1

Not significant -ns= $p>0,05$; significant - *= $p<0,05$; very significant - **= $p<0,01$

The highest degree of correlation between observation parameters is shown between DL1 and SP1 and is 0.947, and the lowest between MY2 and MFC1 0.006. The degree of correlation between milk yield and milk fat content is negative at -0.251. This means that as milk yields increased, milk fat content decreased relatively. In the second lactation the correlation between milk yield traits and milk fat content was not significant ($p>0.05$). The correlation coefficient between CI and SP1 was very significant (** = $p<0.01$), so as the duration of the service period increased, the calving interval extended. The reason is that the service period itself is part of the calving interval, while the other part is the duration of the gestation.

Conclusion

The analysis of the impact of reproductive and milk performance parameters of Holstein-Friesian cows in this paper, which included a total of 364 Holstein-Friesian cows reared on 4 farms of the Agricultural Corporation of Belgrade (PKB) which calved over four seasons in three years the following conclusions are drawn:

- The highest average milk yield in the first and second lactations was in the spring season, and the lowest in the autumn for the first lactation and in the summer for the second.
- The lowest average of the first service period occurred in season 4; the average of the second service period occurred in season 3; the average longest service period occurred in season 2.
- The milk performance traits tested have higher average values in the second than in the first lactation, and the same with the fertility traits.
- Overall, considering the analyzed population of Holstein-Friesian cows, it is possible to continue to improve reproductive traits with a positive effect on all production traits of the cows by applying selection.

Uticaj pojedinih paragenetskih faktora na osobine plodnosti i mlečnosti krava

Ivan Ćosić, Dragana Ružić Muslić, Nevena Maksimović, Bogdan Cekić, Dragan Nikšić, Nenad Mičić, Miloš Marinković

Rezime

Osnovni cilj odgajivačko - selekcijskog rada u mlečnom govedarstvu jeste odabir grla sa genetskim predispozicijama za visoku proizvodnju kvalitetnog mleka, koja svoj potencijal mogu da ispolje u adekvatnim uslovima držanja i koja su po svojim proizvodnim performansama superiornija od prethodnih generacija. Na fenotipsku ispoljenost osobina mlečnosti mogu da utiču brojni faktori, različitog trajanja i intenziteta. Osobine plodnosti dobijaju sve veći značaj u većini savremenih programa za genetsko unapređenje mlečnih goveda, budući da sve više utiču na proizvodnju mleka. Dugotrajana jednostrana selekcija u cilju poboljšanja osobina mlečnosti imala je negativan uticaj na ispoljavanje reproduktivnih svojstava. U analizu uticaja reproduktivnih parametara i parametara mlečnosti

krava holštajn-frizijske rase uključeno je ukupno 364 krava gajenih na 4 farmi Poljoprivredne korporacije Beograd (PKB). Teljena su kroz četiri sezone (proleće, leto, jesen i zima) i to u period od tri godine (2013-2015). Analizom su obuhvaćene sledeće osobine plodnosti: prvi servis period (SP1), naredni servis period (SP2) i međutelidbeni interval (MI), dok su posmatrane osobine mlečnosti uključene kao: prinos mleka prvi (MY1) i naredni (MY2), zatim sadržaj mlečne masti prvi (MFC1) i naredni (MFC2), i još trajanje laktacije prethodne (DL1) i naredne (DL2). Ispitivanje osobine mlečnosti imaju više vrednosti proseka u drugoj nego u prvoj laktaciji, isti trend je zabeležen i kod osobina plodnosti.

Ključne reči: holštajn-frizijska rasa, reproduktivni pokazatelji, osobine mlečnosti, krave

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PRODUCTION PERFORMANCES AND HERD BOOK OF SIMMENTAL AND HOLSTEIN FRIESIAN CATTLE IN CENTRAL SERBIA

Nenad Mičić, Miloš Marinković, Vlada Pantelić, Dragan Nikšić, Marina Lazarević, Nikola Molerović, Ivan Ćosić

Institute for Animal Husbandry, Belgrade – Zemun, 11080 Zemun, Serbia
Corresponding author: Nenad Mičić, micicnenad@hotmail.com
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Abstract: Central Serbia has a long tradition of cattle farming and, as a hilly-mountainous area it favors rearing of dual purpose Simmental breed, and to a lesser extent, Holstein-Friesian dairy breed. The first Herd Book of Serbia was founded in 1935. The high quality breeding animals registered are under the control of production performance properties. According to expert report of the Institute for Animal Husbandry and the results of the implementation of the breeding program in 2018 in the territory of Central Serbia, there were a total of 153.979 quality breeding animals of Simmental and 16509 quality breeding animals of Holstein-Friesian breed in the main herd. Both breeds are of national interest and are included in the breeding-selection program for dairy cattle that are implemented through the cooperation of breeders and breeding organizations in the field. In Central Serbia, there are 157 registered breeding organizations involved in breeding and livestock recording activities. In this paper, data of the Head breeding organization in cattle farming, the Institute for Animal Husbandry, as well as reports issued by the same entity each year were used. A tabular analysis and a five-year trend are presented according to: the number of recorded animals, primiparous females in linear evaluation, the realized yield of milk, milk fat and protein, as well as the trend of the number of bull dams in the population. Based on the analysis of the results of work on cattle breeding and selection, it can be concluded that some progress has been made in milk production of both breeds. It is important to emphasize that there is a significant effect of mentioned factors on the productivity of entire cattle population in Central Serbia. Systematic work on the breeding and selection of cattle is an important work that should be carried out in high quality, continuously and appropriately in order to achieve maximum effects in cattle production.

Key words: cattle, Herd Book, Central Serbia

Introduction

Livestock production is the leading branch of agriculture in the Republic of Serbia with its two most important sectors - milk and meat production. Central Serbia has a long tradition of cattle farming.

According to FAO reports from 2013, the world milk production has grown by more than 50%, over the past three decades, the main producers being the United States, India and China. According to the same FAO data (2013), in Serbia, in relation to the realized net value of agricultural production, dairy production takes the first place. Often, the existing genetic potential of cows is not fully exploited, so attention should be paid to correcting the effects of paragenetic factors of production and farm management (diet, housing and care, feeding systems, welfare, etc.). Selection for high milk production that is also reflected on reproductive performance traits, involves changes in the genotype, metabolism and hormonal status of cows (Zolt, 2016).

Popović (2014), based on the 2012 Agriculture Census, states that there are a total of 908.102 bovine animals in Serbia. The state, by providing some funding to breeders, aims to improve livestock production.

The objective of implementation of the current breeding program (2015-2019) is to increase milk yield while maintaining satisfactory reproductive and fitness traits.

Perišić (2008) states that Serbia had a total of about 1.1 million cattle at that time, with Simmental animals accounting for about 70%.

The establishment of the Central Livestock Union in 1892 and the Agricultural Cooperatives in 1894 contributed to first recording/registration of cows in the form of main records of the breeding animals of the highest quality in Serbia, at that time mainly herds of imported populations of the Simmental breed. The first Herd Book of Serbia was founded in 1935.

Material and Methods

The paper presents the production performances of cattle in Central Serbia, as well as the main goals of the Breeding Program. The data available to the Head Breeding/Selection Organization on the territory of Central Serbia, the Institute for Animal Husbandry, as well as the reports issued by the Institute each year were used. The report on the results of the implementation of the Main Breeding Program from the past 2018 defines the basic selection measures, procedures and methods of selection, their implementation and controls the implementation of

these measures for each breed (GOP, 2015-2019). There is a pyramidal system of stakeholders: from the primary ones performing direct measurements and sampling on animals, through the subsequent ones distributed according to administrative districts of the country, to the head organization providing the results and data analysis and producing the final report to the competent Ministry. Currently, there are 157 registered basic breeding organizations in the territory of Central Serbia that are engaged in activities of animal records and selection of cattle according to the instructions and in cooperation with the head organization and the Herd book.

The methodology for controlling numbers, productivity and other results is in accordance with the provisions of the Law on Livestock production of the Republic of Serbia (*Livestock Law*, 2009, 2012, 2016) and the rules of the International Committee for Agricultural Data Calculation (ICAR, 2014), and these are detailed in the GOP. The Herd Book contains data on the total number of animals of known origin, followed by the data on newly recorded and previously recorded/registered cattle, and information on registered bulls whose semen is used for the fertilization of cows. The control of productivity of the home population of cows implies the results on the achieved production of milk, milk fat and milk proteins, as well as on the linear evaluation of primiparous heifers. The data obtained are analyzed by the statistical program StatSoft.Inc (2010), while the breeding values are determined by the Blup-Animal Model (SPSS 20).

Results and Discussion

In the bovine breed structure of cattle in the Republic of Serbia it is estimated that the Simmental breed makes up about 75%, the group of Black and White Holstein-Friesian cattle about 15%, while fattening and indigenous breeds and crosses make up about 10% of the total number of cattle (HBOCP, 2019). Animals that have been linearly evaluated according to the Instruction for linear type and fitness evaluation of cattle (*Pantelić 2010*, presented at the Seminar of Breeding Organizations of Serbia), with regularly calving, and of known origin for at least two generations of ancestors (*Law on Livestock*, 2009, 2012, 2016) are selected for main herd. In 2013, the share of registered cattle (cattle under control) in the Simmental bovine population was 34% or 86% of the total number of registered cattle of all breeds (HBOCP, 2014).

According to the Herd Book data presented in Ttable 1, for the past five years and the area of Central Serbia, without Vojvodina, there is a trend of increase in the number of heads of both cattle breeds. In her paper, *Ostojić-Andrić (2015)*

reports similar results of a positive trend in cattle selection over a longer period of observation (2005-2014).

Table 1. Number of registered animals under control of both breeds during five year period (2014-2018)

Breed	Number of heads under control				
	2014	2015	2016	2017	2018
Simmental	107860	120861	133926	147957	153979
Holstein-Friesian	16767	17138	17141	17192	16509

The natural resources of the Republic of Serbia are defined by a significantly higher share of Simmental animals (about 80%), especially in the hilly and mountainous regions, i.e. Central Serbia. On the other hand, the Holstein-Friesian breed is mainly reared in the lower regions of Serbia - Vojvodina, while in Central Serbia it represents 10% of the total number of cattle. Although the Holstein-Friesian breed has a relatively lower share in the overall national breed structure, its productive performance as a specialized dairy breed is considered to be an important contributor to the dairy sector in Serbia (*Ostojić-Andrić et al., 2017*).

Linear evaluation of type and body development is very important information on the total breeding value of an animal, on the basis of which breeding heads are selected and classified into classes, and also entered in the Herd Book and obtain the "HB number". Determination of the average value of exterior traits, especially immeasurable properties, is useful information when defining a breeding goal and formulating an effective plan and program (*Lazić et al., 2015*). A linear evaluation of the body conformation enables identification of the traits of dairy animals, which are preliminary indicators of milk yield and longevity. In addition, it indicates the reproductive capacity of the animal, which is of great importance from the point of view of economic efficiency of milk production (*Pantelić et al., 2006*). For the Simmental population, the breeding objective defines the height of the withers of adult animal of over 140 cm and body weight over 650 kg. In addition, the preferable fitness traits, functional traits and animal temperament are also important part of the breeding objectives, followed by favorable calving ease, disease resistance and an average production life of five lactations. *Kebede and Kolmosi (2015)* also conclude that a linear evaluation of the animal is necessary to predict the contribution of cattle performance in programs to improve commercial milk production.

The data in Table 2 shows the results of linearly assessed primiparous heifers of two breeds which after the first calving and linear assessment are included in the main herd, and the trend of change in the number of heads is also shown.

Table 2. Number of primiparous heifers in linear evaluation over a five-year period (2014-2018)

Breed	Number of primiparous heifers in linear evaluation				
	2014	2015	2016	2017	2018
Simmental	16473	22311	19367	25903	24977
Holstein-Friesian	3401	3808	3796	3889	4060

According to the FAO public data (2019) presented in Table 3, total milk production in Serbia is 1550704 tonnes in total and has increased slightly compared to the previous five year period.

Table 3. Total milk production and number of dairy heads (FAO stat data 2019)

Year	Dairy heads	Milk produced (t)
2013	446924	1494412
2014	456477	1536808
2015	431761	1546216
2016	426224	1548697
2017	429595	1550704

Table 4 shows the milk production performance of dairy cows in the past period 2014-2018. Milk production during the reporting period is characterized by a minimal positive trend, where the milk yield of the main population of the Simmental and Holstein-Friesian breeds in Central Serbia is at a nearly constant level with a slight variation interval.

Data on average milk yield of Holstein-Friesian cows for e.g. 2016 is lower than in Croatia (7.633 kg) and Slovenia (7.535 kg) (CAA, 2016; ICAR, 2016a). In Austria, the average milk yield of Holstein cows in 2016 was 8.809 kg with 4.07% milk fat and 3.30% milk protein (ICAR, 2016b). According to WHFF (2016), HF

populations in Israel and the US with yields in excess of 11.000 kg have the highest milk production in the world, with the highest yields in Europe in Denmark, Germany and the Czech Republic (9-10,000 kg). In Serbia, milk production of this breed is at an average level of about 7.000 kg (Table 4).

Based on the results achieved and the assessed possibilities, the breeding objective for the period 2015-2019 foresees an increase in milk yield in the domestic Simmental breed population to an average production of 6.000 kg of milk with a minimum of 4.10% milk fat and a minimum of 3.60% milk protein, and for the Holstein-Friesian breed, the same program anticipates an increase for average production to 8.000 kg of milk with a minimum of 4.00% milk fat and a minimum of 3.50% milk protein (*GOP, 2015-2019*).

Table 4. Production of milk, milk fat and protein of both breeds over a five-year period (2014-2018)

Year	Breed	Number of completed lactations	Milk yield (kg)	Milk fat yield (kg)	Milk fat content (%)	Milk protein yield (kg)	Protein content (%)
2014	HF	10978	7310	264	3.62	236	3.22
2015		11893	7251	258	3.55	229	3.16
2016		11111	7060	253	3.59	221	3.13
2017		11907	6895	252	3.66	219	3.18
2018		11096	6891	254	3.68	221	3.21
2014	SIM	52789	4741	188	3,94	154	3,22
2015		67619	4773	190	3,97	153	3,21
2016		76864	4713	187	3,97	151	3,21
2017		84213	4810	190	3.97	153	3.19
2018		89135	4829	190	3.98	153	3.20

European Union (EU) standards stipulate milk quality and total bacterial count of 100,000 bacteria/ml and somatic cell counts of 400,000/ml, while the current average in Serbia for the total bacterial count in milk is 900,000/ml and somatic cell counts equal to 400,000/ml.

For the purposes of production of domestic bulls of both breeds, the best cows are selected as bull dams, which are at least two standard deviations above

the average of the controled population in milk production, but also according to body development and origin (*Pantelić et al., 2009*). For optimal selection results, it is necessary that these animals make up about 1% of the population, but in Central Serbia this proportion is significantly lower (*Pantelić et al., 2011; Pantelić et al., 2005*).

The number of bull dams is shown in Table 5, accounting for less than 1% of the cow population in both breeds.

Table 5. Number of bull dams of both breeds in the five-year period (2014-2018)

Year	2014	2015	2016	2017	2018	2014	2015	2016	2017	2018
Breed	SIM					HF				
Number of heads	413	410	365	426	410	168	135	102	129	203

Conclusion

The main problem in the current situation in cattle farming in Serbia is the number of heads per farm. Small holdings dominate with less than 3 heads accounting for 50%, while the share of holdings with 20 or more heads is only 3.2% (HBOCP, 2014).

Lately, the colonization of cities has led to the villages being abandoned, accompanied by the unfavorable economic situation in the country. Mountainous regions are less populated and people are moving to lower regions of the country (*Petrović, 2015*).

The number of dairy cows in Central Serbia under control is increasing slightly, despite the decline in the total number of cattle in the country.

According to the European Union (EU) standards set for milk quality, in Central Serbia, there is a trend of decrease in the total bacterial count to less than 100,000 bacteria/ml and less somatic cells than 400,000 bacteria/ml milk.

Based on the achieved results in the cattle selection and breeding, over the past five year period, it can be concluded that some progress has been made in milk production in both breeds. However, given the breeding objective set for the controlled population of cows of both breeds in Central Serbia, breeding objectives (6000kg for SIM and 8000kg for HF) are unlikely to be reached by the end of 2019.

The number of bull dams should be maintained at the level of up to 1% of the highest quality animals on the total population.

Significant efforts have been made in the area of selection to improve the genetic potential of animals, but for optimal results it is also necessary to improve the conditions of feeding, housing and health care, which is difficult under the current socio-economic conditions.

Bearing in mind that milk production is very important as a strategic sector of agriculture in the Republic of Serbia, it is necessary to continue the proper implementation of the set and forthcoming breeding programs for both breeds. An important innovation in breeding programs is the inclusion of major non-productive traits (health, fertility, life expectancy, etc.).

Considering the existing problems in cattle breeding, it is important to consistently implement measures to improve the genetic potential of cattle breeds (Simmental and Holstein-Friesian) in line with the measures foreseen by the agricultural development strategy.

Proizvodni rezultati i matična evidencija goveda simentalске i holštajn rase u Centralnoj Srbiji

Nenad Mičić, Miloš Marinković, Vlada Pantelić, Dragan Nikšić, Marina Lazarević, Nikola Molerović, Ivan Ćosić

Rezime

Centralna Srbija ima dugu tradiciju govedarske proizvodnje i kao brdovito područje pogoduje gajenju kombinovane simentalске, a u manjoj meri i mlečne holštajn-frizijske rase. Prvi *Herd Book* Srbije osnovan je 1935. godine. Kvalitetna priplodna grla registrovana u glavnoj matičnoj evidenciji i knjizi nalaze se pod kontrolom proizvodnih svojstava. Prema stručnom izveštaju Instituta za stočarstvo i rezultatima sprovođenja odgajivačkog programa u 2018. godini na teritoriji Centralne Srbije, ukupno je bilo 153979 kvalitetnih priplodnih grla simentalске i 16509 kvalitetnih priplodnih grla holštajn-frizijske rase u matičnom zapatu. Obe rase koje su od nacionalnog interesa uključene su u odgajivačko-seleksijske programe goveda za proizvodnju mleka koji se sprovode kroz saradnju odgajivača i odgajivačkih organizacija na terenu. U Centralnoj Srbiji postoji 157 registrovanih osnovnih odgajivačkih organizacija koje se bave poslovima matične evidencije i selekcije goveda. U ovom radu korišćeni su podaci glavne odgajivačke organizacije u govedarstvu, Institut za stočarstvo, kao i izveštaji koje isti subjekat izdaje svake godine. Prikazana je tabelarna analiza i petogodišnji trend grla prema:

brojnosti umatičenih grla, linearno ocenjenih prvotelki, ostvarenom prinosu mleka, mlečne masti i proteina, kao i trend broja bikovskih majki u populaciji. Na osnovu analize ostvarenih rezultata rada na selekciji goveda, može se zaključiti da je postignut određeni napredak u proizvodnji mleka obe rase. Važno je naglasiti da se pomenuti efekti reflektuju na produktivnost čitave populacije goveda u Centralnoj Srbiji.

Sistematski rad na odgajivanju i selekciji goveda je značajan posao koji treba da se sprovodi kvalitetno, kontinuirano i u odgovarajućem obimu, kako bi se postigli maksimalni efekti u govedarskoj proizvodnji.

Ključne reči: goveda, glavna matična evidencija, Centralna Srbija

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STUDY OF THE EFFECTS OF LOW TEMPERATURES ON THE MORPHOLOGICAL STATUS OF RAM SPERMATOZOA

Madlena Andreeva¹, Nikola Metodiev², Bogdan Cekić³, Rossen Stefanov¹

¹Institute of Biology and Immunology of reproduction "Acad. K. Bratanov", BAS, Sofia, Bulgaria

²Institute of Animal Science, Kostinbrod, Bulgaria

³Institute for Animal Husbandry, Belgrade, Serbia

Corresponding author: Madlena Andreeva, madlena_andreeva_@abv.bg

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Abstract: The aim of the study was to evaluate how the process of cryopreservation influenced the morphological status of ram sperm. The experiment was carried out with 26 sexually matured, pure bred rams during their breeding campaign. The animals were divided in two groups according to the breed – 15 rams from Synthetic Population of Bulgarian Milk (SPBM) and 11 rams from Sofia breed (SB). The sperm morphology was evaluated under microscope (Nikon Eclipse E200, Japan) by staining with SpermBlue (Microptic, Spain), performed according to the manufacturer's protocol, and observed under bright field with magnification of $\times 40$. Cryopreservation of semen straws was done by the method of Cassou (1964). After cryopreservation, the percentage of spermatozoa with normal morphology decreased and those with abnormal morphology increased. This difference was more pronounced at rams from SPBM compared to SB rams. After freezing, the percentage of spermatozoa with damage to the head at rams from both breeds increased. The percentage of the spermatozoa with damage in the midpiece and the tail was higher and significant ($P < 0.001$) for the SPBM rams. The low temperatures of cryopreservation had a detrimental effect to the rams' sperm structures. This damaging effect was much stronger for the rams from SPBM breed compared to rams from Sofia breed.

Key words: ram, cryopreservation, spermatozoa, morphology status

Introduction

The ability to cryopreserve spermatozoa from all of the domestic species is challenging. Even though all of the cells must endure similar physical stresses

associated with the cryopreservation processes, sperm from the different species are very different in size, shape and lipid composition, all of which affect cryosurvival. Thus, when a cryopreservation protocol has been optimized for sperm of one species, it may not be ideal for sperm of other species (*Purdy, 2006*). Sperm preservation protocols vary among animal species owing to their inherent particularities that change extenders used for refrigeration and freezing. In small ruminants, individual variations in the quality of frozen semen have been observed, suggesting specific differences in sperm susceptibility to freezing methods, (*Barbas and Mascarenhas, 2009*).

During the freeze-thawing process, mammalian sperm are exposed to temperature changes that lead to physical and chemical stress, changes in the plasma membrane lipid composition (*Pérez-Pé et al., 2002*) and externalization of phosphatidylserine residues (*Paasch et al., 2004*). These sperm alterations are dependent on the maturity of the cell (*Host et al., 1999*), the cryoprotectant used (*Soylu et al., 2007*), and the cooling and freeze-thawing rates (*Glander and Schaller, 1999*). Many aspects of sperm protection in the freezing-thawing process, e.g. sperm motility, viability and membrane stabilisation of the sperm cells during relative cryopreservation, are the key factors in determining the preservation of sperm function (*Uysal and Bucak, 2007*). The cryopreservation process acts as an apoptotic inducer in ram semen; some cryoprotectants allowed apoptosis to some extent, with negative effects on sperm morphology and DNA integrity (*Nur et al., 2010*). Semen cryopreservation had little or no effect on the susceptibility of ram sperm DNA to denature in situ when measured immediately at thawing or after 3 hours of incubation, but significant DNA damage appeared later in physiological conditions (*Peris et al. 2004*).

The aim of the study was to evaluate how the process of cryopreservation influenced the morphological status of ram spermatozoa.

Materials and Methods

The experiment was carried out with 26 sexually matured, pure bred rams during their breeding campaign. The animals were divided in two groups according to the breed – 15 rams of Synthetic Population of Bulgarian Milk (SPBM) breed and 11 rams of Sofia breed. The animals had normal sexual performance, aged between 2 – 4 years. Rams from SPBM were raised in experimental base of Institute of Animal Science –Kostinbrod. They were fed with meadow hay ad libitum and concentrated mix (bought from forage company with 12% crude protein) in dose 700 g per ram per day. Rams from Sofia breed were raised in private farm, located in village Mirokovo, Sofia district. They were fed with meadow hay ad libitum and barley grain in dose 500 g. per ram per day. The ejaculates were obtained by the artificial vagina method.

The sperm morphology was evaluated under microscope (Nikon Eclipse E200, Japan) by staining with SpermBlue (Microptic, Spain), performed according to the manufacturer's protocol, and observed under bright field with magnification of $\times 40$. The study was performed before freezing and after thawing of the ejaculates in a specialized laboratory of the Institute of Biology and Immunology of Reproduction.

The cryopreservation was performed at the Institute of Biology and Reproduction Immunology. Cryopreservation of semen straws was done by the method of *Cassou (1964)*. After assessment, the ejaculates normozoospermia were diluted with colloid diluent (6AG), containing cryoprotectant glycerine and then equilibrated. After equilibration the semen were divided in plastic straws (tubes, 133 mm long, 2 mm in diameter and volume of 0, 25 cm³). The one end of the straw is closed with powder of polyvinyl alcohol, placed between two layers cotton-paper filter, and the other end was sealed. The cryopreservation of the straws was conducted on the vapor of nitrogen, and after that they are placed in containers with liquid nitrogen (-196°C) for long term preservation. Before their usage, the straws were thawed by pulling them off the container with liquid nitrogen and placing them directly in horizontal position on water bath, with temperature of + 34°C.

All data were calculated by statistical program IBM SPSS 19. The results of the studied parameters were compared with Paired T-test. The significance of the differences between groups was evaluated by t-criterion of Student. Findings were considered statistically significant if $P < 0.05$.

Results and Discussion

The results for normal and pathological spermatozoa were presented in Table 1. The data for the two tested breeds before freezing and after defrosting were compared for the percentage ratio between sperm with normal and abnormal morphology.

Table 1. Percentage ratio between sperm with normal and abnormal morphology

	Normal % Mean \pm SE	Abnormal % Mean \pm SE
SPBM breed before freezing	89,47 \pm 1,47	10,53 \pm 1,47 ***
SPBM breed after freezing	69,00 \pm 2,45	31,00 \pm 2,45 ***
Sofia breed before freezing	88,91 \pm 1,36	11,09 \pm 1,36 NS
Sofia breed after freezing	84,55 \pm 1,90	15,45 \pm 1,90 NS

Note. Significant differences *** at $P < 0,001$, NS – non significant

The data in the table showed that, after cryopreservation, the percentage of spermatozoa with normal morphology decreased and those with abnormal increased. These differences were more pronounced and significant ($P < 0.001$) for rams from SPBM breed and less pronounced and non-significant for rams from Sofia breed.

Percentage ratio between normal sperm and sperm with damage in head, midpiece and tail is shown in Figure 1. The data presented for both tested breeds are also before and after cryopreservation. Pictures of the morphological status of the spermatozoa before and after freezing were presented at Fig. 2 and Fig. 3 (for SPBM rams) and Fig.4 and Fig.5 (for SB rams).

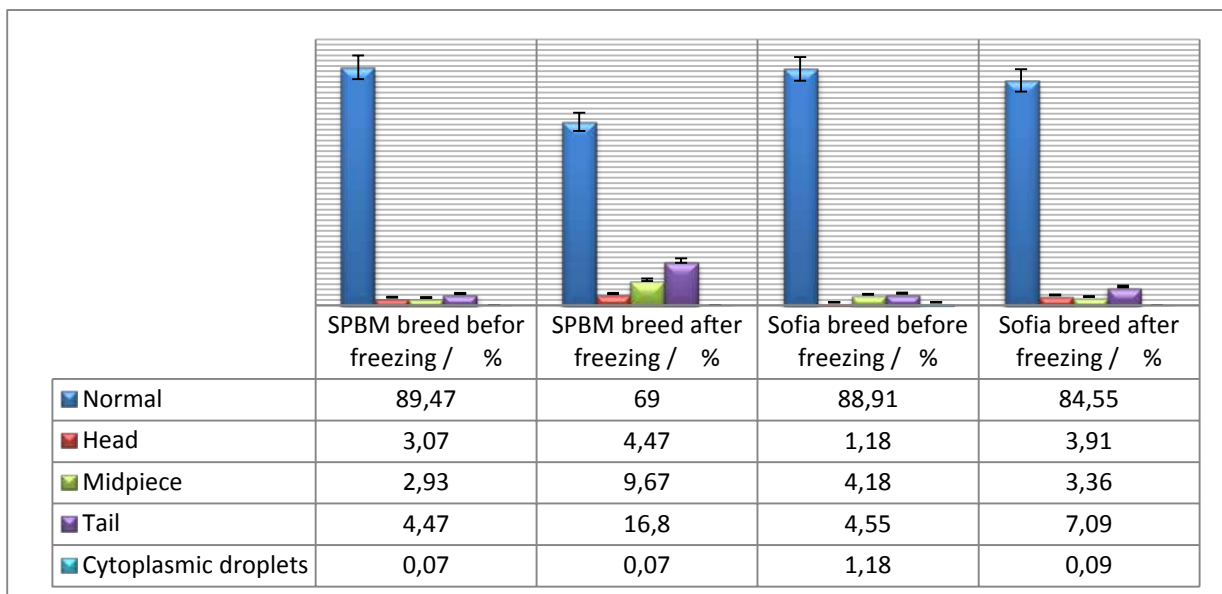


Figure 1. Percentage ratio of spermatozoa with damage in head, midpiece, tail, with the presence of cytoplasmic droplets and normal spermatozoa

The percentages of spermatozoa with damage to the head at rams from both breeds were increased after freezing, but the differences were not significant (Fig. 1). The percentage of affected spermatozoa with damage of the midpiece and tail was higher and significant ($P < 0.001$) after cryopreservation at SPBM breed (2.93% vs. 9.67 for midpiece and 4.47 vs. 16.80 for tail). The same tendency was observed for SB rams, but the differences were not significant (Fig.1).

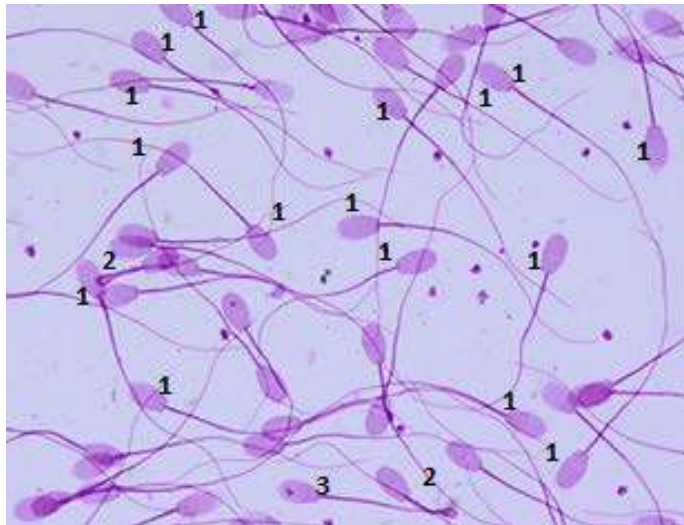


Figure 2. Morphological status of SPBM breed before freezing

- 1 .Normal spermatozoa
- 2 Damage in midpiece
- 3. Damage in tail

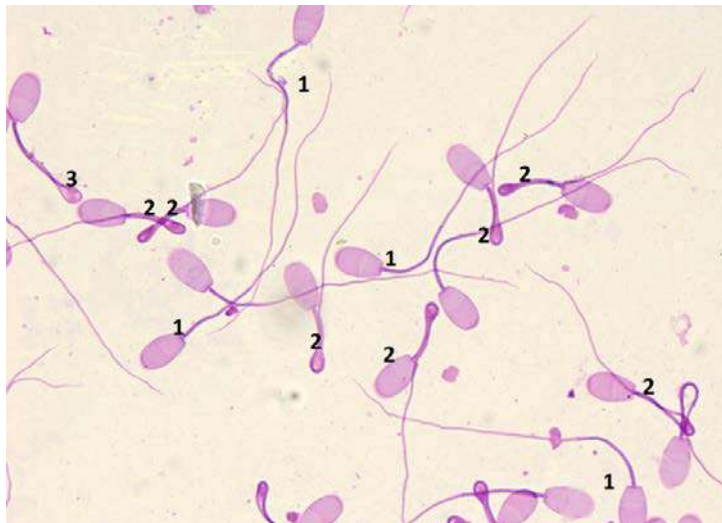


Figure 3. Morphological status of SPBM breed after freezing

- 1 .Normal spermatozoa
- 2 Damage in midpiece
- 3. Damage in tail

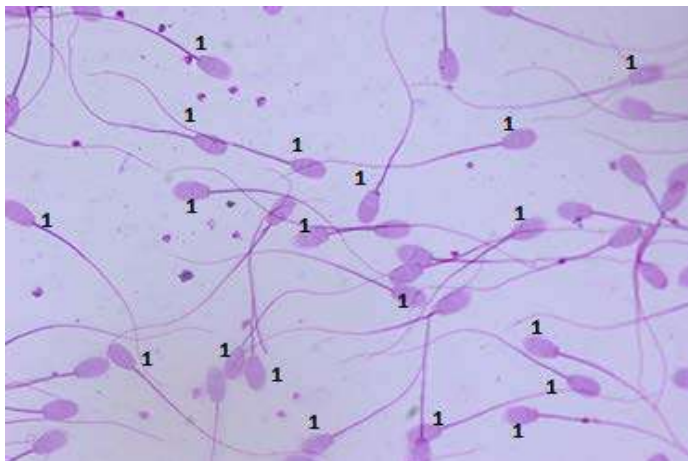


Figure 4. Morphological status of Sofia breed before freezing

- 1 .Normal spermatozoa
- 2 Damage in midpiece
- 3. Damage in tail

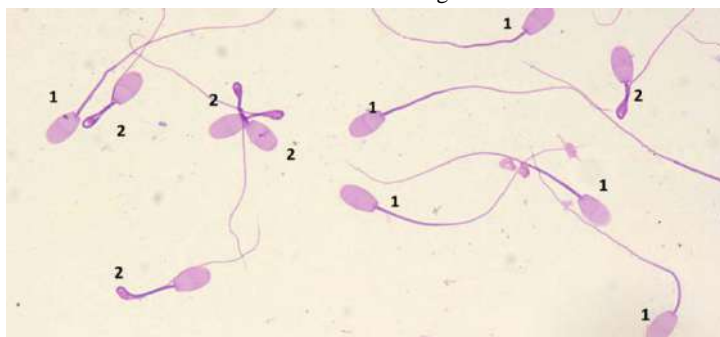


Figure 5. Morphological status of Sofia breed after freezing

- 1 .Normal spermatozoa
- 2 Damage in midpiece
- 3. Damage in tail

According to O'Connell (2002), who have conducted human sperm studies, freeze-thawing caused a 37% reduction in normal morphological forms of sperm. In our study, the reduction of normal sperm after cryopreservation was lower - 20% for SPBM rams and below 4% for SB rams (Table 1).

In other species of animals such as bulls it was found that, the percentage of spermatozoa with normal acrosomes remained higher after dilution, cooling, or equilibration ($73.2\% \pm 2.4\%$) than after freezing and thawing ($61.8\% \pm 2.4\%$; $P < .05$) (Rasul *et al.*, 2001).

Kim et al. (2011) also found the damaging effect of low temperatures. In their study with semen from boar, they found that freezing–thawing may induce reductions in sperm function with increase in membrane damage.

Conclusion

The low temperatures of cryopreservation had a detrimental effect to the rams' sperm structures. This damaging effect was much stronger for the rams from SPBM breed compared to rams from Sofia breed.

Ispitivanje uticaja efekata niskih temperatura na morfološki status spermatozoida ovnova

Madlena Andreeva, Nikola Metodiev, Bogdan Cekić, Rossen Stefanov

Rezime

Cilj rada bio je da se proceni kako je proces krioprezervacije uticao na morfološki status spermatozoida ovnova. Ogled je sproveden na 26 reproduktivno zrelih ovnova čiste rase tokom sezone parenja. Životinje su, na osnovu genotipa, podeljene u dve grupe: 15 ovnova iz Bugarske mlečne sintetičke populacije (Synthetic Population of Bulgarian Milk - SPBM) i 11 ovnova iz Sofijske rase (Sofia breed - SB). Morfologija spermatozoida je procenjena pod mikroskopom (Nikon Eclipse E200, Japan) bojenjem sa SpermBlue (Microptic, Španija), izvedenim prema protokolu proizvođača, i posmatrano pod svetlosnim mikroskopom sa uvećanjem od 40 puta. Krioprezervacija pajeta izvršena je metodom po Cassou (1964). Posle krioprezervacije, procenat spermatozoida sa normalnom morfologijom se smanjio, a onih sa abnormalnom se povećao. Ova razlika je bila izraženija kod ovnova iz SPBM u poređenju sa SB ovnima. Posle zamrzavanja, povećan je procenat spermatozoida sa oštećenjem glave kod ovnova obe rase. Procenat spermatozoida sa oštećenjem u sredini i repu bio je veći i značajniji ($P < 0,001$) za SPBM ovnove. Niske temperature krioprezervacije imale su štetan efekat na strukturu spermatozoida ovnova. Ovaj štetni efekat bio je mnogo jači za ovnove SPBM rase u odnosu na ovnove SB rase.

Ključne reči: ovnovi, krioprezervacija, spermatozoidi, morfološki status

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MONITORING AND TRENDS FOR DEVELOPMENT OF SREDNORHODOPSKA SHEEP

Tsonka Odjakova, Pavel Todorov, Atanaska Zgurova

Research Center of Stockbreeding and Agriculture - Smolyan
Corresponding author: Tsonka Odjakova, tsonkaat@abv.bg
Original scientific paper

Abstract: Monitoring of Srednorhodopska sheep from Smolyan region was presented analyzing exterior indicators and live weight of contemporary population breed compared to the same data described by other studies. 124 exterior measurements have taken, and an assessment of the exterior features was carried out, depending on their historical development, by calculating the exterior indexes. An increase in the size of ewes has observed in comparison to those reared in the mid-20 century. The larger size of contemporary animals is due to improved conditions, climate change, and farmers' desire for more production. The information gathered can be applied to identify the relevant genetic distances in the future.

Key words: srednorhodopska sheep, exterior, population, measurements

Introduction

The Srednorhodopska sheep breed is a common local breed for the region of Western, Middle and Eastern Rhodopes. The sheep have bred in small to medium-size flocks (*Dechev, 1905*). Before the Second World War, a large number of these and other local breeds had been grazing on the high mountain pastures of the Rhodopes Mountain in the summer and in the plains of the Aegean Thrace in the winter. In the book "Pasturage in the Middle Rhodope" *Vasil Dechev* has published, several indicators for the productivity of the breed.

According to *Hlebarov, 1940*, Srednorhodopska sheep has the lowest live weight compared to other local breeds. *Madrov (1936)* also performed exterior body measurements for sheep of various native breeds, including Srednorhodopska sheep.

More particular information about the breed's condition has obtained from *Marinov (1968, 1970, 1973)*. In his works, he made in-depth studies on growth, body shapes, and meat qualities, of the Srednorhodopska sheep. *Vasilev et al. (2000)* examine the growth intensity, milk productivity, and body measurements in the Srednorhodopska sheep.

The assessment of animal by its exterior is of great importance for the tribal work in sheep breeding. Animal measurement is an accurate and objective method.

Hinkovski et al. (1984) wrote that the exterior is one of the main selective signs alongside the type and constitution of the animals. For this purpose, established standards for body measurements have used, where average values of the measurements of different points of the body are specified, serving for the objectivity of the expert assessment.

According to *Raichev et al. (1992)*, by body measurements, the body sizes of a given animal or group of herds of a known breed were determined. Body measurements can also serve to track the development of individual parts of the body of the animals for a longer period of time. The exterior is one of the main indicators of selection and is a reflection of animal productivity.

The aim of this study was to characterize the Srednorhodopska sheep population state at present compared to the available information from other authors.

Material and methods

The subject of the researches is the Srednorhodopska sheep grown in the region of Smolyan region, situated in Western Rhodops in Bulgaria.

Breed Description

The first data on the breed has published by *V. Dechev (1905)*, namely the live weight of sheep - 25kg, of ram - 40kg, one-year-old weaned lamb - 23kg. The average wool yield from the rams was 1,850 kg per animal, from the ewes - 0.950kg. During the milking period is obtained an average of 12-15 kg milk. The sheep's wool is rough.

The data received in the study gave similar descriptions of Srednorhodopska sheep. It is one of the smallest of our native breeds. The head is of medium length, delicate, with an almost straight profile line. The forehead is moderately broad. The eyes are lively and expressive. The ears are medium sized and properly placed. The lips are thin and highly mobile. The face is white, but in many individuals, there are darker or lighter spots - splotch-faced, blondes, spotted, etc. The rams are usually with spiral horns. Females rarely develop horns, but when present, the sheep are called rogushi. Male animals without horns also exist and are called cabaci. The neck of Srednorhodopska sheep is relatively thin and medium in length. The dorsal line is almost straight, but not quite horizontal, gradually rising to the waist. The chest is narrow and quite shallow. The belly is comparatively bulky as a result of eating relatively large amounts of rough and green fodder from

a young age. The tail is short, thin, reaching up to and below to hock joints. The udder is small, with medium-sized teats. The legs and hooves have underlined strength. The fleece of most of the animals is open or semi-open, with a fuselage construction and coarse wool (cabarlyavi), but there are animals with almost closed fleece (rudavi). About 60-70% of the sheep are white and about 30-40% brown, grey and black. The Srednorhodopska sheep can refer to short- and thin-tail sheep breeds. It is closer to the Zackel than to the Tsigai. It is highly mobile, can travel long distances and use grazing in highly sloping areas, unavailable to other farm animals. It has a highly developed herd instinct, emphasized endurance and adaptability. In general, Srednorhodopska sheep is a combined type of type for wool, meat and milk.

Methods

The obtained data from the current study will be compared to the data published from other authors - *Madrov (1936)*, *Marinov (1968, 1970, 1973)*, *Vasilev et al. (2000)*.

123 ewes at 2.5 years of age had measured in 2017 for the live weight and body characteristics - height at withers, chest depth, rump width, body length, chest girth. The measurements had done Lydtin's rod and Vilken's compass. The live weight has obtained with scale. Indices for leg length, stretching, chest, pelvic-chest, massiveness, and compactness were calculated. The summarized results are presented in tables and diagrams.

Results and Discussions

Live weight

The live weight data for the Srednorhodopska sheep published by different authors shown in Table 1.

Table 1. Live weight in kg of females' sheep as compared to data from other authors.

Items	Madrov (1936)	Marinov (1973) n 1985	Measurements (2017) n 123
Live weight ewes	26.7	23.18	39.65

The live weight was lowest for the animal described in 1973 (23.18 kg). Our data shows the highest live weight (39.65 kg). The results obtained by *Madrov (1936)* and *Marinov (1968)* confirms the claims that the Srednorhodopska breed has the lowest live weight. The trend is the same by comparing our data to the published one for Srednostaroplaninska sheep (*Genkovski 2002*).

Body measurements

Measurements done in 2017 have compared to previously published data for the breed from the same region with similar agroecological features.

Table 2. Body measurements in Srednorhodopska sheep, compared to data from other authors.

Items	Madrov (1936)	Marinov (1973) n 1985	Measurements (2017) n 123
Wither height, cm	54.45	54.58	63.98
Height of rump, cm	56.67	56.55	-
Diagonal body length, cm	59.27	56.79	74.49
Chest depth, cm	25.45	22.68	28.89
Chest width, cm	16.73	14.02	19.6
Chest girth, cm	71.18	67.68	81.63
Rump width, cm	-	13.3	19.16
Rump length	14.93	18.92	-
Tail length, cm	24.5	25.57	-

Body measurements in 2017 have performed on animals classified in the T class (typical for the breed). The height at the withers of the Srednorhodopska sheep was almost the same in 1936 and 1973, respectively 54.45 and 54.58 cm. Our sheep are 9.53 centimeters higher than those measured by *Madrov (1936)* and 9.4 centimeters of that measured by *Marinov (1973)*.

The sheep body length of Srednorhodopska breed, measured by *Marinov (1973)*, is 2.48 cm shorter than that of *Madrov (1936)*. Significant differences were observed in modern representatives compared to the 1936 and 1973 studies, respectively by 20.43% and 23.76% respectively.

The differences in the depth of the chest indicator follow the same trends. The animals measured by *Marinov (1973)* were 22.68 cm against 25.45 cm by *Madrov (1936)*. The Srednorhodopska sheep (2017) have a more developed chest, as the differences being respectively more with 3.44 and 6.21 cm compared with data obtained in 1936 and 1973.

The differences in the width of the chest in different groups are greater. The difference between contemporary representatives is respectively higher, with 14.64% and 28.47%, compared to the data from *Madrov (1936)* and *Marinov (1973)*.

For chest girth, the highest values were registered in animals measured in 2017 - 81.63 cm. The data published in 1936 and 1973 were similar, respectively 71.18 and 67.68 cm. The rump width is bigger with 5.86 cm in the Srednorhodopska sheep measured in 2017.

Madrov (1936) analysed 14 breeds and established the lowest body measurements in the Srednorhodopska sheep. The trend is repeated in the studies of *Marinov (1973)*, comparing the results with those for Karakachan, Karnobat and local sheep from Strandja.

Our study indicates that contemporary sheep have higher average values for body measurements than those published in the past. It is clear from the presented results that our sheep show some similarity but also significant differences with the ones studied in the past. The differences attributed to improved nutrition and rearing of contemporary sheep, as well as the spontaneously running gene drift.

Body indexes

Indexes are a relationship between two or more body dimensions associated anatomically or functionally, expressed in %. They characterize the proportions of the body and change during the growth and development of the animals. Through them, growth has controlled, deviations from the norm- embryo and infantilism have established. For a more complete characterization of the exterior of the Srednorhodopska sheep in the study period, we calculated the indexes of leg length, stretching, chest, pelvic-chest, compactness and massiveness. The results are presented in figures.

The Srednorhodopska sheep studied by *Marinov (1973)* are a bit higher than *Madrov's studies (1936)* and our data from 2017 (Figure 1).

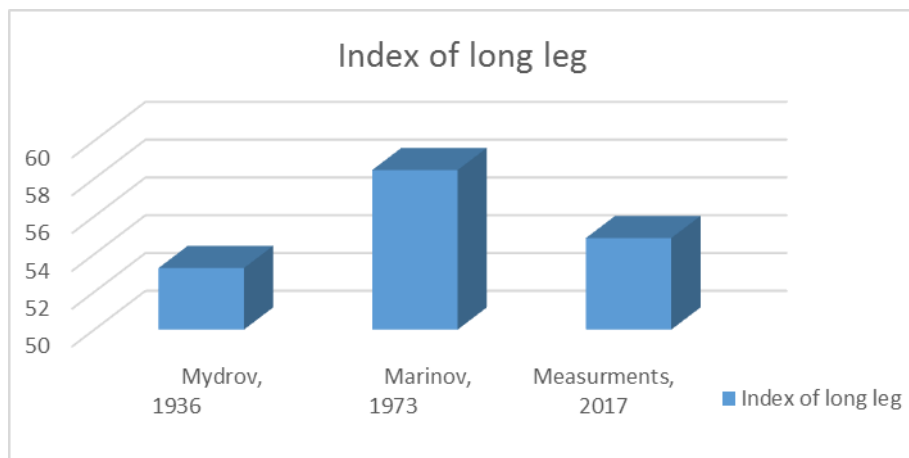


Figure 1. Index of long leg

The index of body stretching expresses the ratio between body length and height and is calculated by dividing the length of the body into the height at the withers (x 100). The index varies very little with age. The results in figure 2 show that the most stretched are the animals measured in 2017 - 116%. The values are close in *Madrov* (1936) and *Marinov* (1973), respectively 109 and 104%.

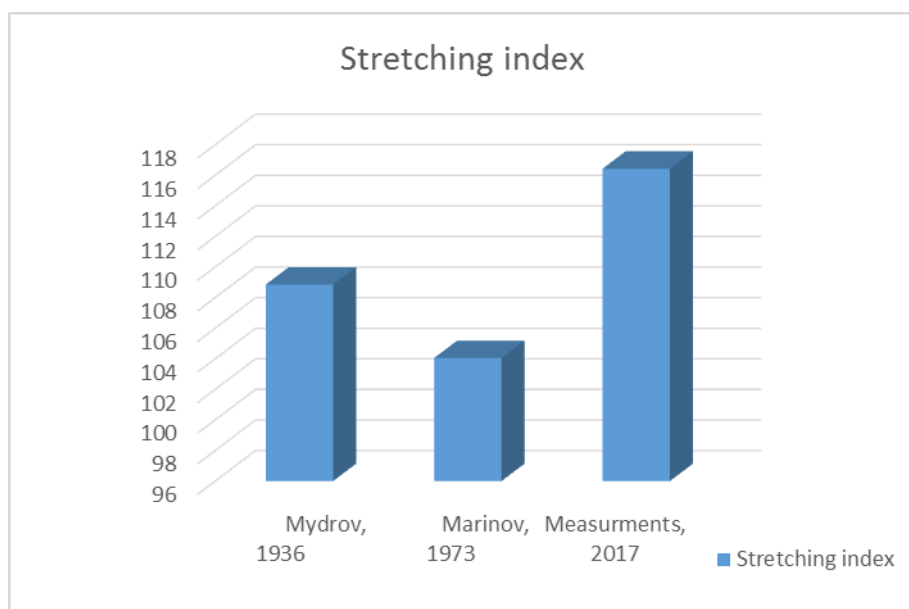


Figure 2. Stretching index

The chest index shows the relative development of the chest, its width and barrel-shaped. The current data for the index - 68% - is higher than those published in 1936 and 1973, respectively 66% and 62%, and confirms the results obtained. The chest index of the Srednorhodopska sheep is close to that of the Srednostaroplaninska (68.18%) published by *Nedelchev and Panayotov (2014)*.

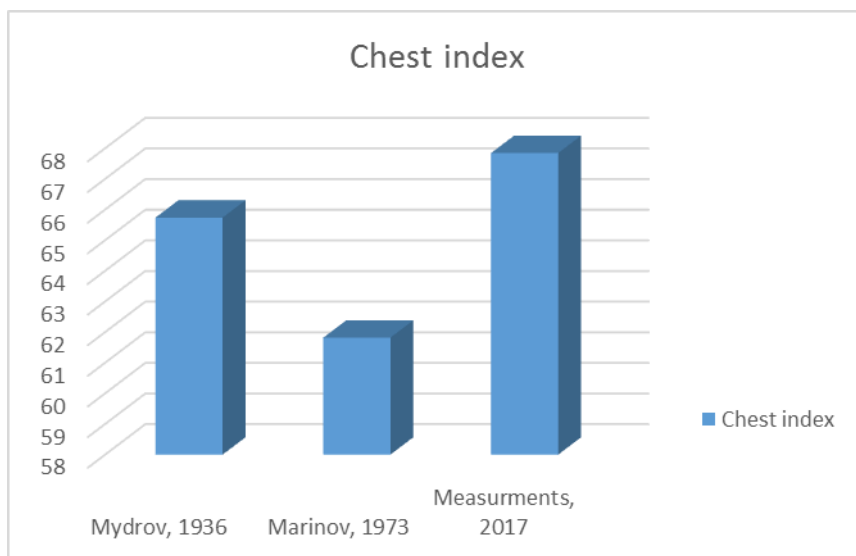


Figure 3. Chest index

The relative development of the chest width compared to the pelvis shows no difference in the study period. The results obtained are close to 105 and 102%, respectively, in 1973 and 2017 (Figure 4).

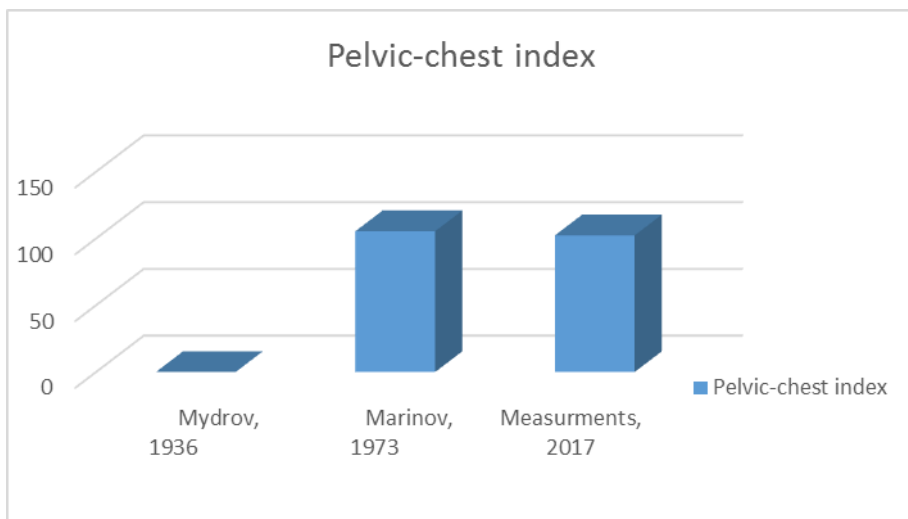


Figure 4. Pelvic-chest index

The massiveness index indicates a relative development of the body. Recent results and those published in the past are similar in value and show that the average sheep are comparatively aligned, 124% *Marinov (1973)* and 128% in 2017 research.

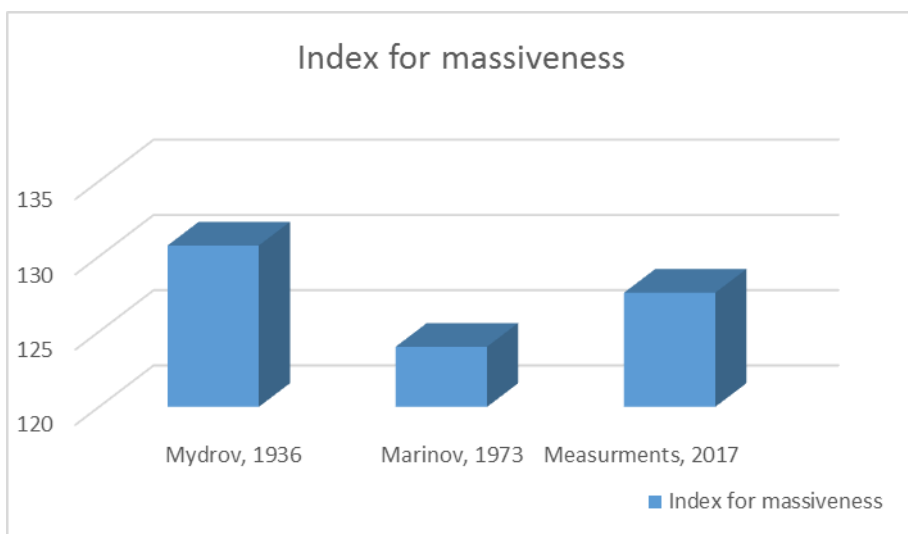


Figure 5. Index for massiveness

The index of compactness is calculated as ratio of the chest girth and body length (x 100). This index is a good indicator of body weight and characterizes body compactness, supplementing the chest index. The lowest values are 110% for our animals. This result in the Srednorhodopska sheep is close to that of the Srednostaroplaninska breed (126.88%) published by *Genkovski (2002)*.

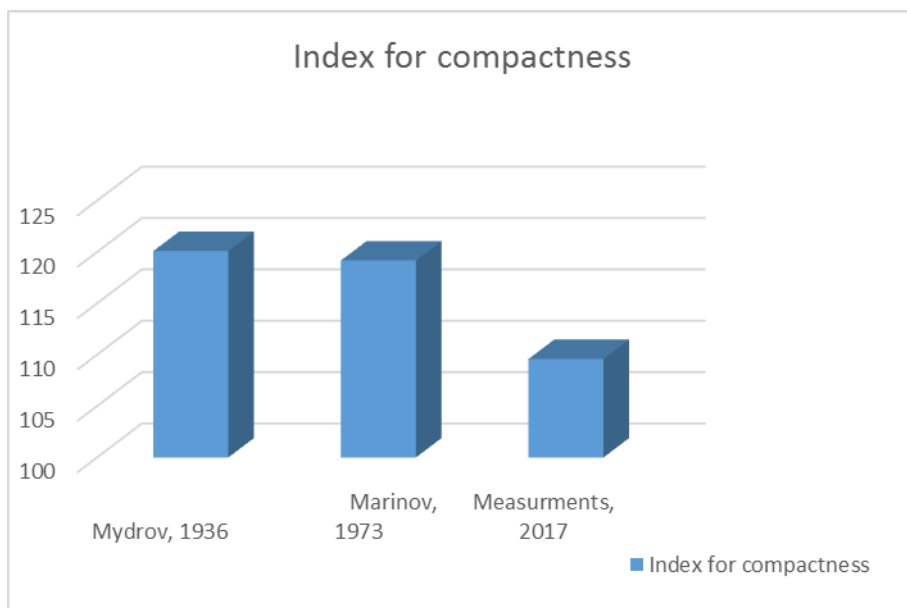


Figure 6. Index for compactness

Conclusions

Our study on Srednorhodopska sheep shows that, according to morphological features, the animals are similar to those breed in the past and classified as indigenous pigmented sheep. The contemporary representatives of the Srednorhodopska sheep show height at the withers of 63.98 cm, body length - 74.49 cm, chest depth - 28.89 cm, chest width of 19.6 cm, chest girth - 81.63 cm and rump width - 19.16 cm. The assessment of the exterior according to the historical development, and on the basis of the calculation of the body indexes give us an idea on how the consolidation of Srednorhodopska breed is going. The larger size of contemporary animals is due to improved conditions, climate change, and farmers' desire for more production. The information gathered can be applied to identify the relevant genetic distances in the future.

Monitoring i trendovi razvoja srednje rodopske ovce

Tsonka Odjakova, Pavel Todorov, Atanaska Zgurova

Rezime

Prikazan je monitoring srednje rodopske ovce iz regiona Smoljana, kroz analizu spoljašnjih pokazatelja i žive mase savremene populacije ove rase u poređenju sa istim podacima koje su opisali *Madrov (1936)*, *Marinov (1973)* i *Vasilev i sar., (2000)*. 124 eksteriorne mere su uzete i ocenjene, u zavisnosti od njihovog istorijskog razvoja, izračunavanjem indeksa eksteriornih osobina. Primećen je porast veličine ovaca u poređenju sa onima koje su uzgajani sredinom 20 veka. Veća veličina savremenih životinja rezultat je poboljšanih uslova, klimatskih promena i želje farmera za većom proizvodnjom. Prikupljene informacije mogu se koristiti za identifikaciju relevantnih genetskih udaljenosti u budućnosti.

Ključne reči: srednje rodopski ovca, eksterijer, populacija, merenja

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DIFFERENT OESTRUS SYNCHRONIZATION PROTOCOLS IN LACTING NORTH-EAST BULGARIAN MERINO SHEEP IN ANESTRAL PERIOD

Rossen Stefanov¹, Georgi Anev², Madlena Andreeva¹, Plamen Todorov¹, Nevena Maksimović³

¹Institute of Biology and Immunology of Reproduction, BAS, Sofia, Bulgaria

²Experimental station of Agriculture, Targovishte, Bulgaria

³Institute for Animal Husbandry, Zemun, Belgrade, Serbia

Corresponding author: Rossen Stefanov, stefanovrossen@gmail.com

Original scientific paper

Abstract: The aim of the present study was to compare three schemes for synchronization of oestrus in lactating sheep in the anestrus period, using megestrol acetate (MGA), pregnant mare serum gonadotropin (PMSG) and prostaglandin F_{2α}. The experiment was done with 150 lactating sheep of North-east Bulgarian merino breed divided into three groups, aligned by age, live weight and physiological state. The animals of all three groups received 4 µg of megestrol acetate, dissolved in 95% ethyl alcohol and carefully mixed with concentrated feed for 8 days. On the 10th and 24th day of the beginning of the experiment, the sheep of all three groups were injected with 500 UI of PMSG. Animals in groups II and III additionally received PGF_{2α} in the dose of 167 µg, which was administered 24th day in group II and 10th and 24th day in group III. Sheep in oestrus were detected in the morning and evening using teaser rams for 8 days from the beginning of each cycle. The fertility rate was calculated by the number of lambs born. Treatment of sheep with PMSG caused the stretching of oestrus period of 4-5 days. Incorporation of PGF_{2α} and its analogues into the treatment regimen produced a good synchronic effect, which is to thicken the infiltration within 2 days. Injection of PGF_{2α} only during the second treatment resulted in the same synchronicity effect.

Key words: sheep, serum gonadotropins, progesterone, oestrus synchronization

Introduction

It is known that with the use of progestogens and serum gonadotropins, physiologically adequate ovulation in sheep may be induced during seasonal anestrus (*Boland and Kelleher, 1978; Bartlewski et al., 1998; Barrett et al., 2004; Gonzalez-Bulnes et al., 2005*). It also has been found that the efficiency of insemination during the first oestrus after treatment with progestogens and PMSG is unsatisfactory and it is therefore recommended that it be carried out during the second cycle (*Menchaca and Rubianes, 2004*). Using this synchronization protocol, sheep show signs of oestrus for 3-4 days after treatment, making it difficult to practice artificial insemination. This has led to the search for other schemes for oestrus synchronization. Suitable prostaglandin $F_{2\alpha}$ and its analogues have a rapid luteolytic effect (*Acritopoulou et al. 1982; Beck, and Williams, 1993; Viñoles et al., 2011; Fierro et al, 2013*). In most animals, oestrus comes between 29 and 48h after treatment with prostaglandin $F_{2\alpha}$ when injected 24h prior to gonadotropin treatment.

The aim of the present study was to compare three schemes for synchronization of oestrus in lactating sheep of the investigated North-east Bulgarian merino breed tested in the anesthetic period using megestrol acetate (MGA), pregnant mare serum gonadotropin (PMSG) and prostaglandin $F_{2\alpha}$.

Materials and Methods

The experiment was carried out in the sheep farm of the Experimental station of agriculture, Targovishte on 150 lactating sheep of North-east Bulgarian merino breed divided into three groups, aligned by age, live weight and physiological state. Number of sheep by groups was as follows: I – 58 sheep – of age 2 years old and weighing 43-47 kg, II – 40 sheep – of age 2, 5 years old and weighing 47-53 kg and III – 52 sheep – of age 3 years old and weighing 53-57 kg.

The animals of all three groups received 4 µg of megestrol acetate, dissolved in 95% ethyl alcohol and carefully mixed with concentrated feed for 8 days. On the 10th and 24th day of the beginning of the experiment, the sheep of all three groups were injected with 500 UI of PMSG. Animals in groups II and III additionally received PGF $_{2\alpha}$ in the dose of 167 µg, which was administered 24th day in group II and 10th and 24th day in group III.

Sheep in oestrus were detected in the morning and evening using teaser rams for 8 days from the beginning of each cycle. Oestrus of sheep was monitored

for three cycles from the beginning of the experiment. Thereafter, both the onset and the duration of the induced oestrus were recorded.

During the first oestrus the sheep were registered without being inseminated. During the next oestrus sheep were vaginally artificially inseminated with fresh semen, diluted with diluent 6A, twice in a range of 7-8h. In the third cycle, the newly discovered sheep and the repeats from second cycle were inseminated.

All data were calculated by statistical program IBM SPSS 19. The results of the studied parameters were compared with Paired T-test. The significance of the differences between groups was evaluated by t-criterion of Student

Results and Discussion

Oestrus synchronization efficiency expressed by percentage of sheep showing oestrus symptoms in the first cycle is shown in figure 1.

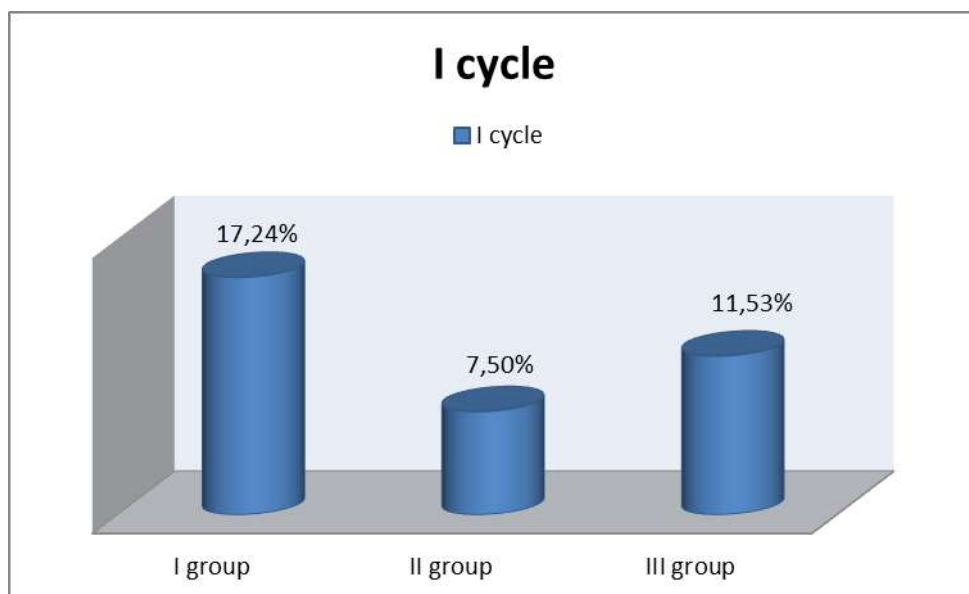


Figure 1. Percentage of sheep showing symptoms of oestrus in the first cycle

The data from figure 1 shows that symptoms of oestrus during the first cycle manifested in 17.24% of sheep in group I, 7.5% of sheep in group II and

11.53% of sheep in group III. Prostaglandin $F_{2\alpha}$ treatment did not result in changes in number of sheep in oestrus in first sexual cycle. Such a result is also found in other similar studies (Taher, 2014).

In the second cycle (figure 2), the number of sheep in oestrus in all three groups was higher, but the difference wasn't significant ($P>0.05$). For group I it was 60.34%, for group II 75% and for group III 69.23%. The greater percentage of sheep in oestrus in groups II and III during the second cycle after the treatment can hardly be explained by the action of the prostaglandin analogue and should be considered as a coincidence. In these groups, however, the synchronizing role of prostaglandin $F_{2\alpha}$ is very well expressed. All sheep of groups II and III showed symptoms of oestrus for the first two days, whereas for group I, symptoms of oestrus were observed for 4 days, making it difficult to program the pregnancy. Therefore, treatment with prostaglandins has led to an acceleration of ovulation. Accelerated ovulation in sheep under the action of prostaglandins is an interesting species feature.

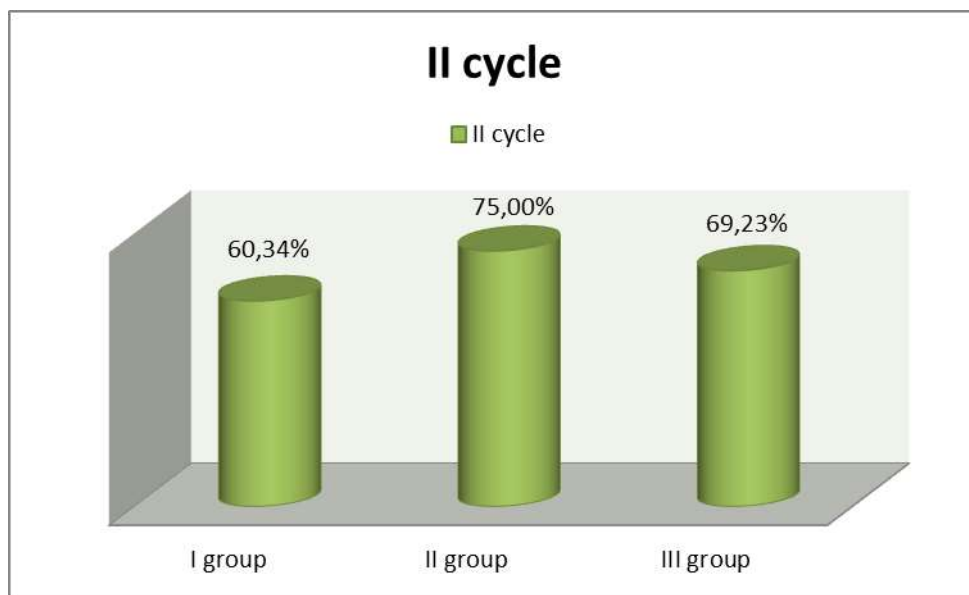


Figure 2. Percentage of sheep showing symptoms of oestrus in the second cycle

To determine the most appropriate time for artificial insemination in the groups II and III, the onset of induced oestrus and its duration were traced. Figure 3 shows the distribution of sheep according to the hours of oestrus after administration of gonadotrophic hormones during the second cycle. In all sheep,

the onset of oestrus occurred between the 28th and the 50th hour after the administration of the gonadotropins. Duration ranges from 18 to 23h. There are no particular differences between the groups surveyed.

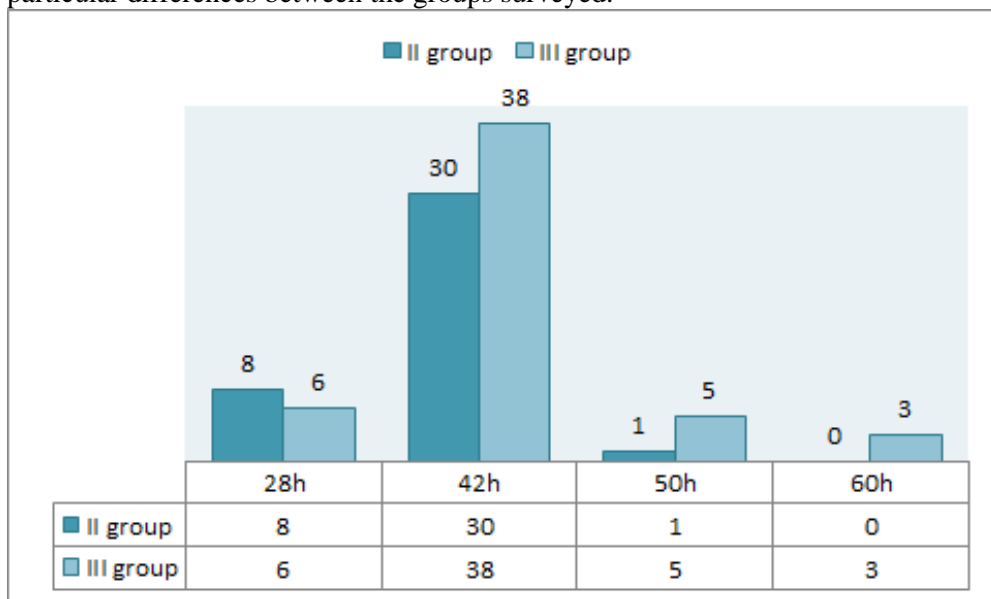


Figure 3. Distribution of ewes by hours of oestrus manifestation

The number of newly discovered sheep in oestrus and reps during the third cycle is within normal limits (figure 4). This indicates good conception from artificial insemination during the second cycle. Percentage of sheep showing oestrus was as follows: I group 55.17%, II group 55% and III group 59.61%. The oestrus response was similar in all three groups. The results obtained justify claiming that the treatment of sheep with prostaglandin F_{2α} does not adversely affect the fertility of the animals.

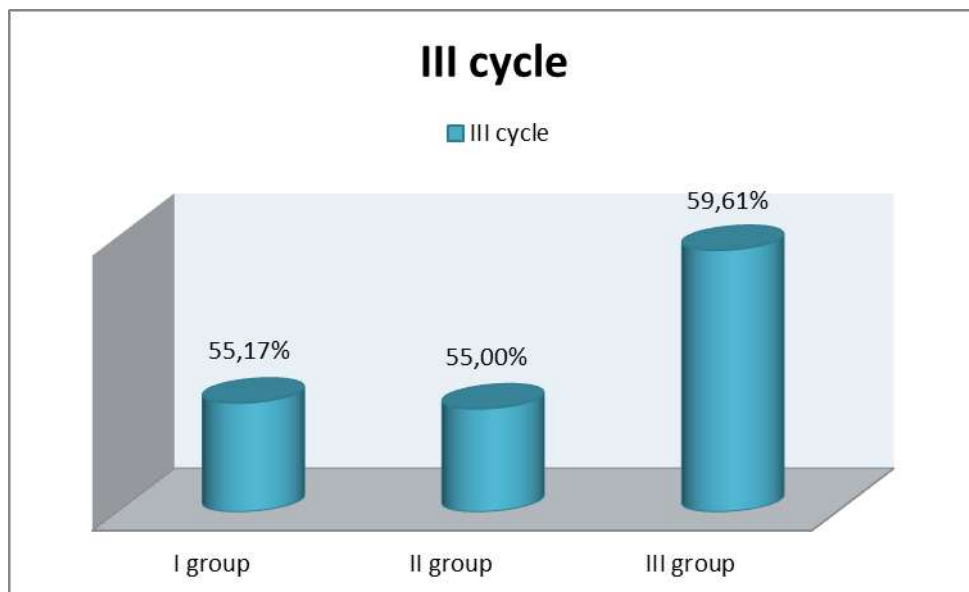


Figure 4. Percentage of sheep showing symptoms of oestrus in the third cycle

One limitation of PMSG is its long-acting biological activity, causing it to continually recruit antral follicles, which results in a large number of unovulated follicles (Armstrong et al., 1983). Another potential limitation of PMSG has been the declining fertility after long-term use. Baril et al. (1996) found PMSG to delay the onset of estrus in goats and this delay in timing of estrus has been suggested as one of the major causes of the reduction in fertility seen in does repeatedly treated with PMSG.

The use of one, two, or more PG injections for flock synchronization depends on the level of synchrony desired, the economics, and the ability of each farmer to manage the flock during a highly concentrated breeding and lambing period (Fierro et al., 2013). Good results have also been obtained with oestrus synchronization in sheep with progesterone-impregnated intravaginal sponges and prostaglandin analogue (Loubser and Niekerk). This confirms our results that prostaglandins and their analogues are appropriate to different oestrus synchronization protocols.

Conclusion

Treatment of sheep with PMSG causes a stretching of oestrus period of 4-5 days.

Incorporation into the treatment regimen of $\text{PGF}_2\alpha$ and its analogues produces a good synchronic effect, which is to thicken the infiltration within 2 days.

Injection of $\text{PGF}_2\alpha$ only during the second treatment results in the same synchronicity effect.

Različiti protokoli za sinhronizaciju estrusa ovaca u laktaciji severoistočne bugarske merino rase u anestralnom periodu

Rossen Stefanov, Georgi Anev, Madlena Andreeva, Plamen Todorov, Nevena Maksimović

Rezime

Cilj ove studije bio je upoređivanje tri šeme za sinhronizaciju estrusa ovaca u laktaciji tokom anestralnog perioda, korišćenjem megastrol acetata (MGA), seruma gonadotropina steonih kobilica (PMSG) i prostaglandina $\text{F}_2\alpha$. Eksperiment je urađen na 150 ovaca u laktaciji, severoistočno bugarske merino rase, podeljenih u tri grupe, prema starosti, živoj masi i fiziološkom stanju. Životinje iz sve tri grupe dobile su 4 μg megastrol acetata, rastvorenog u 95% etil alkoholu i pažljivo pomešane sa koncentrovanom hranom za 8 dana. Desetog i dvadesetčetvrtog dana od početka eksperimenta, ovacima sve tri grupe ubrizgano je 500 UI PMSG-a. Životinje u grupama II i III su dodatno primile $\text{PGF}_2\alpha$ u dozi od 167 μg , koji je primenjen 24. dana u grupi II i 10. i 24. dan u grupi III. Ovce u estrusu otkrivene su ujutro i uveče pomoću ovnova probača tokom 8 dana od početka svakog ciklusa. Stopa plodnosti izračunata je prema broju rođene jagnjadi. Tretman ovaca PMSG-om izazvao je produženje estrusa u trajanju od 4-5 dana. Uključivanje $\text{PGF}_2\alpha$ i njegovih analoga u tretmane stvorilo je dobar sinhroni efekat, a to je zadebljanje infiltracije u roku od 2 dana. Injekcija $\text{PGF}_2\alpha$ samo tokom drugog tretmana rezultirala je istim efektom sinhronosti.

Ključne reči: ovca, gonadotropini u serumu, progesteron, sinhronizacija estrusa

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QUALITY MILK COMPOSITION AND COAGULATION ABILITY IN SHEEP FROM THE BULGARIAN DAIRY SYNTHETIC POPULATION WITH DIFFERENT GENOTYPES

Daniela Miteva, Stayka Laleva, Teodora Angelova, Daniela Yordanova, Nikolay Ivanov

Agricultural Institute – Stara Zagora
Corresponding author: Stayka Laleva, stalajj@abv.bg
Original scientific paper

Abstract: In Bulgaria, a number of studies have been carried out with Bulgarian Dairy Synthetic Population (BDSP) sheep determine the parameters of milk productivity during the different stages of breed creation. The animals of the Bulgarian Dairy Synthetic Population have characterized by very good quality indicators of milk. The present study aim was to analyze the milk quality composition and its individual coagulation ability in sheep from the Bulgarian Dairy Synthetic Population with different genotypes. In the study were examined 306 individual milk samples of Bulgarian Dairy Synthetic Population (BDSP) ewes from the herd of the Agricultural Institute, Stara Zagora. Samples have taken from three consecutive milk controls during the lactation period - April 2016 – June 2016. The best indicators of milk quality and coagulation ability traits had observed in sheep with blood from Stara Zagora and Lacaune sheep breeds. The highest percentage of fatty substances – 7.33% was demonstrated by ewe with 62.5% blood from Bulgarian Dairy Synthetic Population, 12.5% blood of Stara Zagora sheep and 25% blood of Lacaune – 7.33%. Sheep with 75% blood from Bulgarian Dairy Synthetic Population and 25% from Stara Zagora sheep, produced milk, with the hardest coagulum – 50 mm.

Key words: coagulation properties, sheep milk, genotype

Introduction

In Bulgaria, a number of studies were carried out with sheep from the Bulgarian Dairy Synthetic Population (BDSP) to evaluate milk production parameters throughout the different stages of breed creation (*Dzhorbineva et al., 1995; Nedelchev et al., 2003; Raycheva et al., 2003; Hinkovski et al., 2008*). BDSP

sheep are outlined with outstanding milk quality (*Stancheva, 2012*). The main characteristic of sheep milk is high yield of produced cheese per liter milk (*Bencini, 2002*), and milk coagulation properties are important traits in cheese production (*Bertoni et al., 2005; Bittante et al., 2011; Summer et al., 2002*). That is why the improvement of milk coagulation ability of sheep milk is essential for the quality and profitability of dairy productions (*Kalaydzeiv et al., 2012*).

The study aim was to analyze the proximate composition of milk and its coagulation ability in sheep from Bulgarian Dairy Synthetic Population from different genotypes.

Material and Methods

Quality milk composition and coagulation properties have analyzed in 306 individual milk samples collected from Bulgarian Dairy Synthetic Population (BDSP) sheep reared in the flock of the Agricultural Institute – Stara Zagora. Samples had obtained from three consecutive test days during the lactation period (April 2016 – June 2016).

To fulfill the study's goal, the pedigree database of BDSP sheep born between 2006 and 2015 in the flock of the Agricultural Institute – Stara Zagora was analyzed to determine milk coagulation ability and quality milk composition. The genetic code of each animal comprised 8 digits, each consisting codes of both parents. The breeds involved in crossing schedules of the studied flock in the pedigree database of BDSP sheep have coded as followed:

1 – Bulgarian Dairy Synthetic Population (BDSP); 2 – Lacaune (L); 3 – Chios (CH); 4 – East-Friesian (EF); 5 – Stara Zagora breed (SZ); 6 – Pleven Blackhead breed (PB); 9 – the origin of one of the parents is not identified (NI).

Data were analysed with Systat 13 software, a graphs were plotted in EXCEL.

Results and Discussion

Figure 1 presents the results from milk fat content in sheep with different proportion of blood. The parameter varied from 4.71 to 7.33%. The research of *Ivanova et al. (2015)*, showed higher milk fat percentage: from 6.39 to 7.15%.

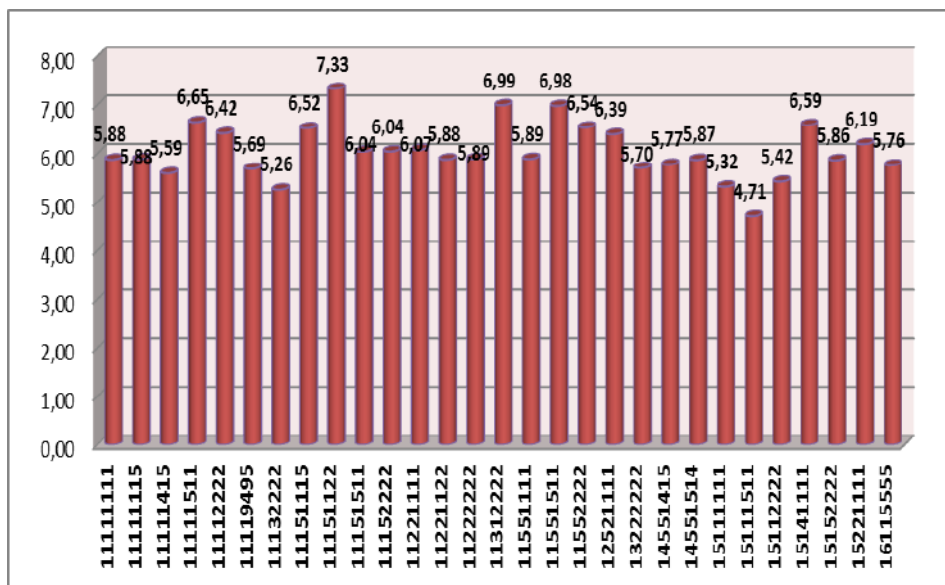


Figure 1. Fat content of BDSP sheep milk

The highest milk fat content (7.33%) had established in animals with 62.5% blood from BDSP, 12.5% SZ blood, and 25% Lacaune blood. Comparable results (7.32% milk fat) was reported also by *Stancheva et al. (2011)* in a study on BDSP sheep in the farm of the Agricultural Institute – Shoumen in 2008.

Animals with genetic codes 11222222 (25% BDSP : 75% Lacaune) and 11551111 (75% BDSP : 25% SZ) exhibited equal milk fat content – 5.89%. This was also observed in sheep with codes 11111111 (100% BDSP), 11111115 (87.5% BDSP : 12.5% SZ) and 11221122 (50% BDSP : 50% Lacaune) – 5.88%. Milk with the lowest fat content (4.71%) was produced by sheep with 62.5% BDSP blood and 37.5% Stara Zagora blood.

Figure 2 depicts the milk protein content of BDSP sheep. The variation of this trait was not as pronounced as milk fat content. Ewes with codes 14551514 (37.5% BDSP : 37.5% SZ : 25% EF) and 11551511 (62.5% BDSP : 37.5% SZ) demonstrated the highest milk protein content: – 5.07% and 5.03%, respectively. Higher values of the studied trait were reported by *Ivanova et al. (2015)* – 5.66–6.05%, and by *Stancheva et al. (2011)* – 5.35%.

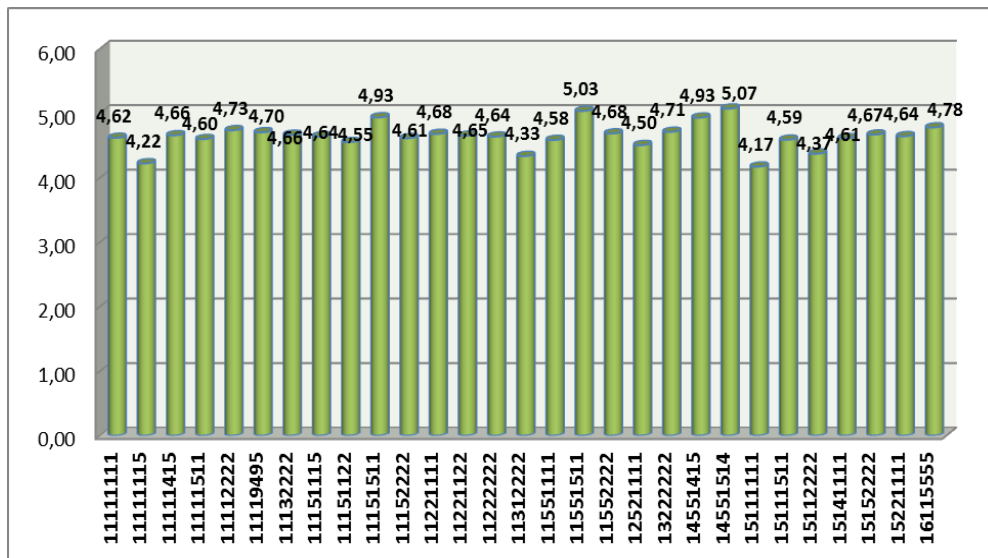


Figure 2. Protein content of BDSP sheep milk

The data obtained for non-fat dry matter (NFDm) in the milk of BDSP ewes are presented in Figure 3. The highest values were shown by animals coded as 11551511 (62.5% BDSP: 37.5% SZ) – 10.64%, 14551415 (37.5% BDSP : 37.5% SZ : 25% EF) – 10.45%, 14551514 (37.5% BDSP : 37.5% SZ : 25 % EF) – 10.30%, 11151511 (75% BDSP : 25% SZ) – 10.23% and 11222222 (25 % BDSP :75% Lacaune) – 10.03%. The variation of the parameter in the other combinations was not marked.

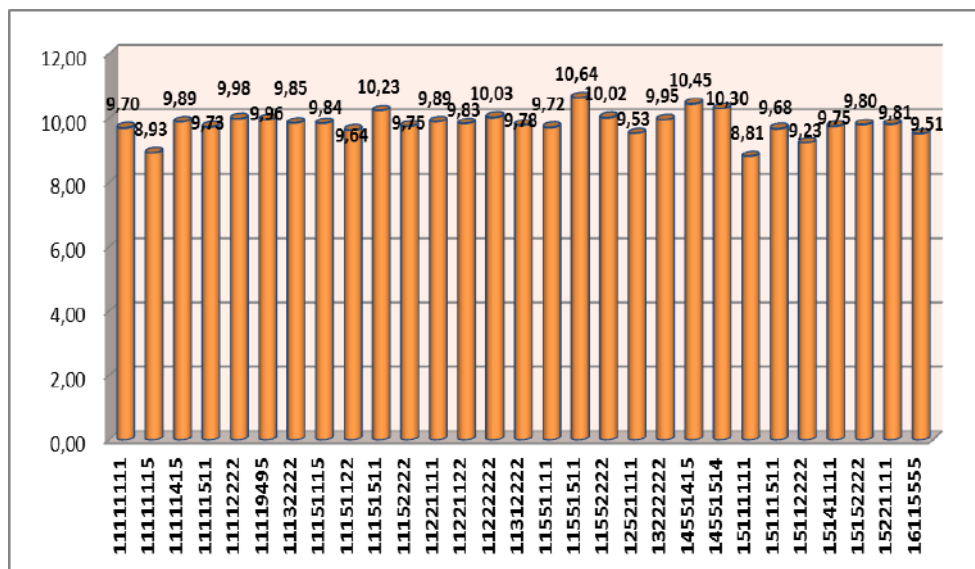


Figure 3. Non-fat dry matter (NFD) content of BDSP sheep milk

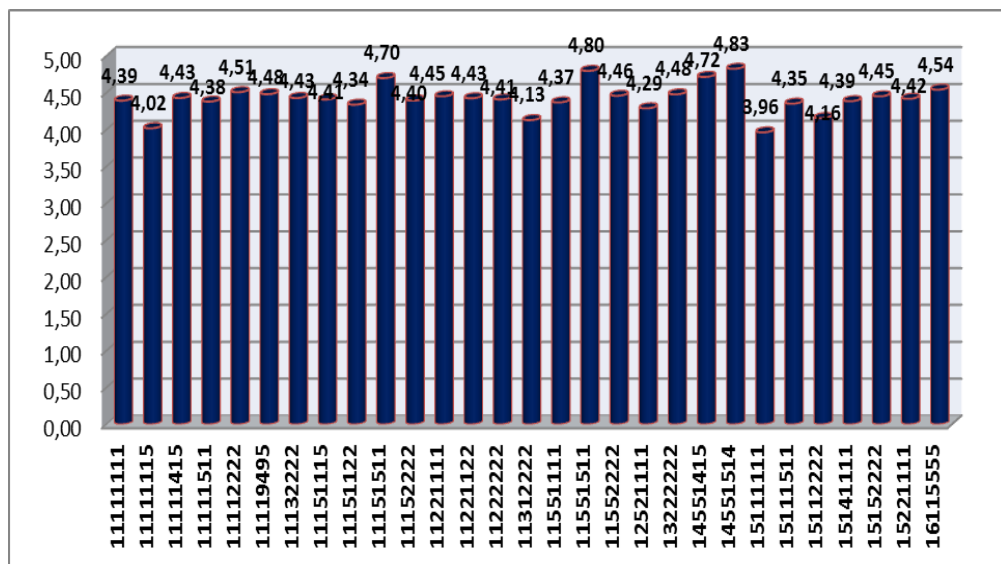


Figure 4. Lactose content of BDSP sheep milk.

Milk lactose content in studied sheep has indicated in Figure 4. The highest lactose percentage was exhibited by ewes coded 14551514 (37.5 % BDSP : 37.5% SZ : 25% EF) – 4.83% and 11551511 (62.5% BDSP : 37.5% SZ) – 4.80%. Sheep with

genetic code 15111111 (87.5% BDSP : 12.5% SZ) are outlined with milk with lowest lactose content – 3,96%. In BDSP sheep reared in the farm of the Agricultural Institute - Shoumen, *Stancheva et al. (2011)* found out milk lactose content of 4.25%.

Figure 5 shows rennet coagulation time of the milk of BDSP ewes. Obtained values varied within a broad range. The shortest RCT was found out in ewes with 37.5% BDSP blood, 50% Lacaune blood, and 12.5% Chios blood – 8.91 min. A relatively short RCT was observed in ewes with codes 12521111 (62.5% BDSP: 12.5% SZ : 25% Lacaune) – 9.93 min, 15221111 (62.5% BDSP : 12.5% SZ : 25% Lacaune) – 10.50 min, 11151115 (75% BDSP : 25% SZ) – 10.75 min. The coagulation of milk was slowest in the milk of ewes coded 15112222 (37.5% BDSP : 12.5% SZ : 50% Lacaune) – 30.00 min, 15111111 (87.5% BDSP : 12.5% SZ) – 30.00 min. An important fact from the technological point of view was the lack of milk samples which did not coagulate within 30 minutes.

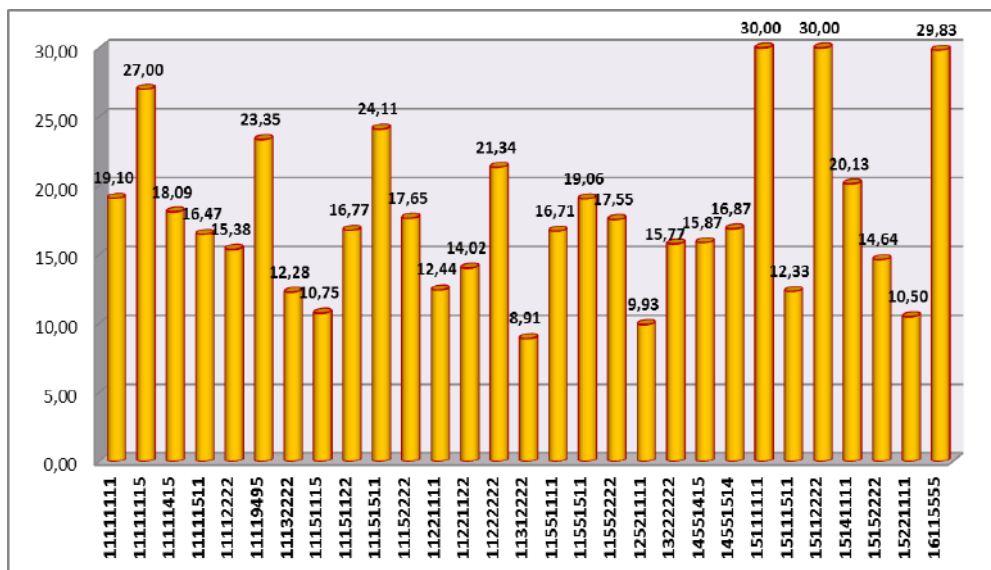


Figure 5. Rennet coagulation time (RCT) of BDSP sheep milk

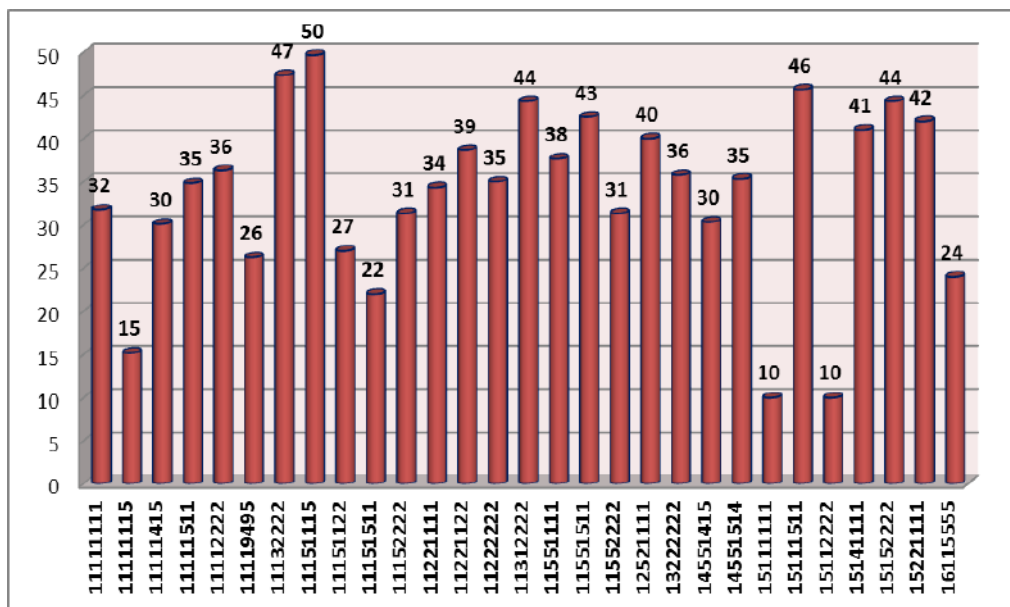


Figure 6. Curd firmness (A₃₀) of BDSP sheep milk

Curd firmness (A₃₀), a parameter characterizing milk coagulation properties, is presented in Figure 6. Ewes with code 11151115 (75 % BDSP : 25 % SZ) were outlined with the firmest coagulum – 50 mm, whereas the loosest one was shown in the milk of 11111115 ewes (87.5% BDSP : 12.5% SZ) – 15 mm, 15111111 ewes (87.5% BDSP : 12.5% SZ) – 10 mm and 15112222 ewes (37.5% BDSP : 12.5 % SZ : 50% Lacaune) – 10 mm. In general, the milk of ewes included in this study, had a relatively firm coagulum.

Figure 7 presents the curd firming time (min) in BDSP ewes. The highest CFT was exhibited by animals coded 11221122 (50% BDSP : 50% Lacaune) – 0.69 min, 11111415 (75% BDSP : 12.5% SZ : 12.5% EF) – 0.65 min and 15141111 (75% BDSP : 12.5% SZ : 12.5% EF) – 0.64 min. The lowest curd firming time, respectively fastest milk coagulation occurred in ewes with codes 15111111 (87.5% BDSP : 12.5% SZ) – 0.20 min, 15112222 (37.5% BDSP : 12.5 % SZ : 50% Lacaune) – 0.20 min, 11151511 (75% BDSP : 25% SZ) – 0.23 min and 11111115 (87.5% BDSP : 12.5% SZ) – 0.24 min.

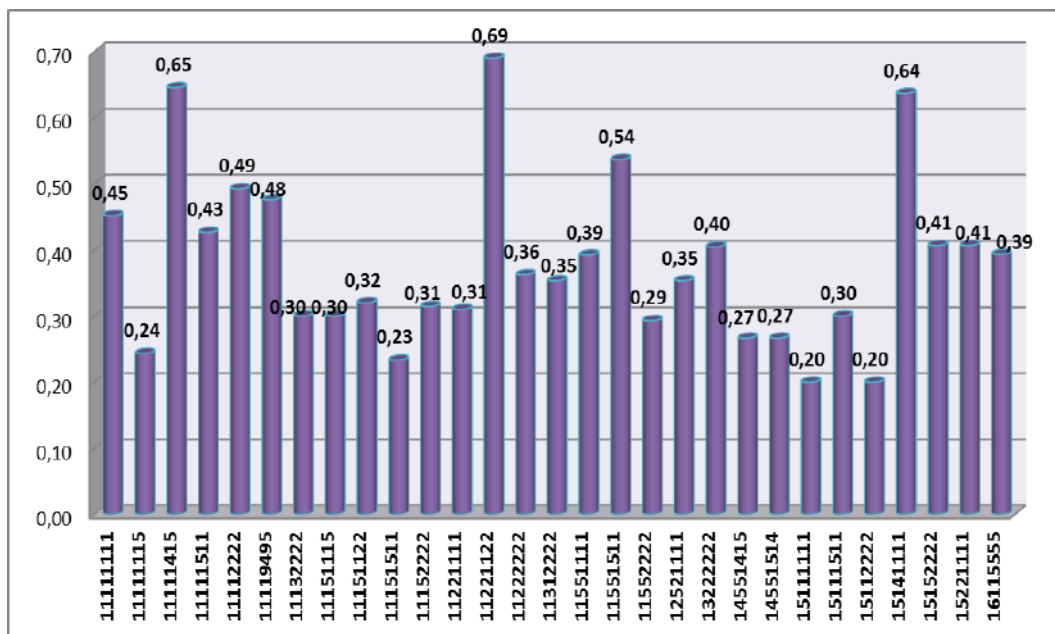


Figure 7. Curd firming time (K₂₀) of BDSP sheep milk

Conclusions

1. The best milk quality and coagulation properties had demonstrated by sheep with blood from Stara Zagora and Lacaune blood.
2. The highest milk fat content had observed in ewes with 62.5% BDSP blood, 12.5% Stara Zagora sheep blood, and 25% Lacaune blood – 7.33%.
3. Sheep with 75% BDSP blood and 25% Stara Zagora sheep blood produced milk, outlined with the firmest coagulum – 50 mm.
4. The sheep with blood from Zagora and Lacaune showed the best milk quality and coagulation properties.

Kvalitetan mlečni sastav i sposobnost koagulacije ovaca Bugarske sintetičke mlečne populacije različitih genotipova

Daniela Miteva, Stayka Laleva, Teodora Angelova, Daniela Yordanova, Nikolay Ivanov

Rezime

U Bugarskoj je sprovedeno niz istraživanja sa ovcima Bugarske sintetičke mlečne populacije (Bulgarian Dairy Synthetic Population - BDSP) koja utvrđuju parametre mlečnosti u različitim fazama stvaranja rase. Životinje Bugarske sintetičke mlečne populacije u Bugarskoj karakterišu veoma dobri pokazatelji kvaliteta mleka. Cilj ove studije bio je analiza kvaliteta mleka i njegove pojedinačne sposobnosti koagulacije kod ovaca iz Bugarske sintetičke mlečne populacije sa različitim genotipima. U istraživanju je ispitano 306 pojedinačnih uzoraka mleka Bugarske sintetičke mlečne populacije (BDSP) iz stada Poljoprivrednog instituta u Staroj Zagori. Uzorci su uzeti iz tri uzastopne kontrole mleka u periodu laktacije april 2016 - jun 2016. Najbolji pokazatelji kvaliteta mleka i osobina koagulacione sposobnosti primećeni su kod ovaca s krvlju rasa iz Stare Zagore i Lacaune. Najveći procenat masnih supstanci - 7,33%, pokazala je ovca sa 62,5% krvi bugarske mlečne sintetičke populacije, 12,5% krvi ovaca Stara Zagora i 25% krvi Lacaune - 7,33%. Ovce sa 75% krvi bugarske sintetičke populacije i 25% Stare Zagore, proizvele su mleko, sa najtvrdim koagulumom - 50 mm.

Ključne reči: koagulaciona svojstva, ovčje mleko, genotip

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THE PIG PRODUCTION IN THE CZECH REPUBLIC - REQUIREMENTS FOR FARROWING MANAGEMENT IN HIGHLY PROLIFERATIVE SOWS

Jaroslava Bělková, Miroslav Rozkot, Eva Václavková

Institute of Animal Science Prague Uhřetín, Department of Pig Breeding, Kostelec nad Orlicí, 517 41 Kostelec nad Orlicí, Czech Republic

Corresponding author: Jaroslava Bělková, belkova.jaroslava@vuzv.cz

Review paper

Abstract: The Czech Republic ranks among the developed European countries at the level of pig reproduction indicators. Despite reduction in sows during 2018, there was practically identical number of born piglets as in 2017. This confirms the dynamics of improving reproduction indicators, reflecting intense breeding work and increasing selection pressure in pig breeding. An increasing number of piglets in the litter is associated with a number of problems, including low birth weight, low uniformity of litter and increased piglet mortality. Therefore, strategies are required to help us reduce losses and optimize large litter breeding. There are higher demands on breeding, technology and management and require increased care for sows and piglets after delivery. In today's highly proliferative sows, it is advisable to use modern technologies that are adapted to the body's framework, behavior, and production intensity.

Key words: pig production, farrowing management, large litters, highly proliferative sows, temporary confinement

Introduction

In the today's challenging times for pig farmers, each piglet represents a chance for increasing sales and thus maintaining the competitiveness of the breeders. However, an increasing number of piglets in the litter is associated with a number of problems, including low birth weight, low uniformity of litter and increased piglet mortality. It also places higher demands on breeding, technology and management and requires increased care for sows and piglets after delivery.

The selection of hyperprolific sows to increase litter size and profit, caused a significant increase in the percentage of low-birthweight piglets up to 23% (*Martineau and Badouard, 2009*). These piglets also show slower growth and deterioration in the parameters of meat and pig carcass quality, resulting in an

economic loss rather than an increased profit. Therefore, strategies are required to help us reduce losses and optimize high-litter breeding. Preventive measures can be carried out in sows already during pregnancy. Maintaining the optimum condition of the sows is of crucial importance, as it helps increase the performance in the lactation period, affects the percentage of cull sows, and helps with normal fetal development. We can optimize the intrauterine environment by enriching sow nutrition. Addition of lipids is advisable, which, according to the new knowledge, reduces the occurrence of low birth weight piglets and improves survival. A balanced amino acid ratio is also important, which increases litter uniformity. L-glutamine and L-carnitine can be added, as they have positive effects on the birth weight of piglets. There are different views on the amount of feed in the last stage of pregnancy. Generally, sows should not be overfed at the last stage of pregnancy to avoid difficult farrowing, MMA syndrome and excessive weight loss during the lactation period. According to some experiments, the diet can be reduced to 1.8 kg/day in the last third of pregnancy without affecting the birth weight of the piglets, with a positive effect on maximizing feed consumption during the lactation period (*Mallman et al., 2018*). In another experiment, different levels of energy were provided to pregnant sows and the weight of sows and piglets was monitored. Maximum piglet weights at birth and weaning were achieved at an energy level of 1.5 maintenance requirements of sows rather than at lower levels (1.0 or 0.5 maintenance requirements) (*Ren et al., 2018*).

Preparing for farrowing

Sows are usually moved to a farrowing crate about a week before farrowing, which is important for handling the sow and so that the sow gets used to the new environment and for adaptation of the immunological profile. Obviously, the farrowing place must be thoroughly washed and disinfected in advance, and dried, if needed, to reduce germ survival as much as possible. Likewise, the feeding system must be cleaned, whether used for solid or liquid feed. Surviving germs often cause mammary gland inflammation in the sow. This can be a major complication in large litters, where every well-functioning teat is welcome. Similarly, bacteria may cause health problems in piglets, which are compromised due to low birth weight and unable to cope with a bacterial infection. Diarrhea is the cause of high mortality, reduced weight gain and other health complications.

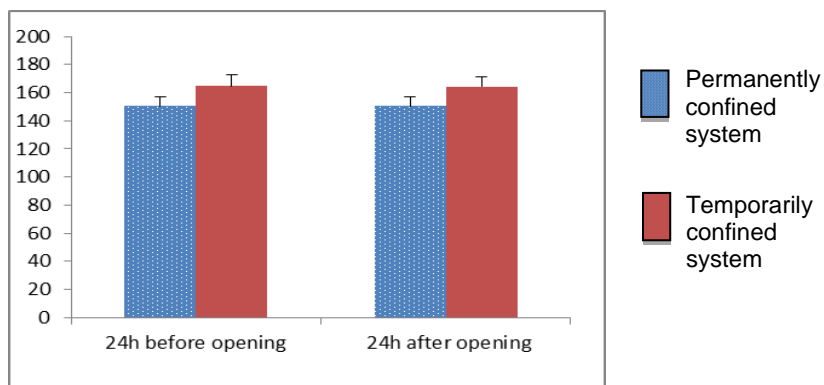
It is also necessary to check the function of the drinkers before placing the sow in the farrowing room. Nursing sows consume 30-40 liters of water per day, and it is essential that the drinker has a flow rate of at least 2.5 liters per minute, more preferably 4 liters per minute, and this should be checked regularly during the lactation period. It is preferable to dispense water directly into the trough, which

makes water intake easier for sows. Water temperature is also significant, and water intake is significantly higher for cold water (10°C) than warm water (25°C).

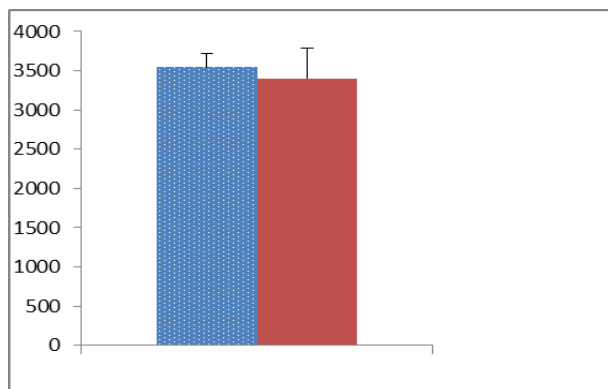
The optimum temperature in the barn for sows is 18-22°C, and 15-25°C is acceptable. If this limit is exceeded, performance is reduced and it is therefore important to check the proper functioning of the air-conditioning system or ventilation. Low temperature is not necessary, however, drafts in the stable should be avoided to decrease the risk of MMA problems. When using the All In All Out system, it is possible to increase the temperature before the expected farrowing to 20-22°C and gradually reduce it during the lactation period to 19-21°C starting on postpartum day 4 up to 18-19°C.

In today's high-fertile sows, it is advisable to use modern technologies that are adapted to the body's framework, behavior, and production intensity in these sows. It is important to provide enough space for high-number litters and to allow good suckling conditions. In relation to the efforts to improve welfare, the traditional cage housing of farrowing or nursing sows is being abandoned. It can be replaced with open-design crates or pens or group housing, providing plenty of space to the animals, unfortunately with possible negative effects, such as higher piglet mortality, higher housing needs and worse conditions for pig keepers. This results in decreased productivity and impaired economic results of pig farming. Temporary crating offers a compromise between the needs of sows and piglets and the requirements of breeders. The new-design pens provide more space and freedom to the sow, while also trying to protect the piglets from crushing (overlying). They provide good suckling conditions by respecting the animal's natural behavior. At Institute of Animal Science Prague, we have been engaged in the development of this type of pens. The research was carried out in project of the Ministry of Agriculture, Czech Republic, QJ1610390 Housing of sows providing welfare and economy. In the experiments, sows were placed in a closed pen (about 1.6 m²) and from day 4, the barriers were opened and the sow had a free pen (4.6 m²). Graph 1 and Graph 2 show the results of weight gain in piglets from sows in temporary crating compared to permanent cage housing (*Illmann, 2019*). Graph 3 shows a comparison of piglet mortality (*Illmann, 2019*). An important finding was that housing of sows in a temporary cage was not associated with a statistically significant reduction in weight gain of piglets or with a significant increase in piglet mortality. The result is the farrowing pen WELLUP (*Rozkot et al., 2018*), which will satisfy the growing demand for higher levels of pig welfare in the Czech Republic. It is a combined housing technology for nursing and farrowing sows, which combines the use of cage fixation of sows for several days with free housing for the remainder of the lactation period. Compared to the conventional technology, the alternative system of crate housing for nursing and farrowing sows are usually burdened with a markedly lower production efficiency at higher

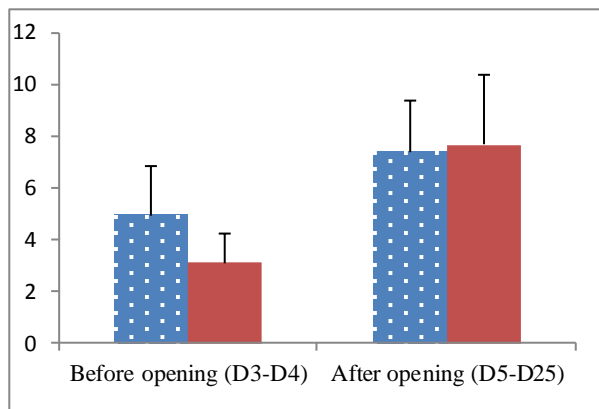
investment and operating costs and the WELLUP pen has been designed to minimize the said negative effects in practice.



Graph 1. Average weight gain of piglets (g)



Graph 2. Weight gain of piglets after 20 days (g)



Graph 3. Total mortality (%)

Digestion and nutrition of sows

It is always necessary to monitor the consistency of excrements in sows. Optimal microflora must be maintained in the gastrointestinal tract, which has beneficial effects on the bodily condition. Its disruption increases the load and negatively affects performance. When the passage of the digestive tract in the intestinal tract slows down, waste metabolites and endotoxins may accumulate, which play an important role in the development of MMA syndrome. It is very important to avoid constipation and maintain the correct consistency of the faeces during the farrowing period. A complete feed for nursing sows should therefore be a source of natural fiber in the form of high-quality fiber components, such as wheat bran, sprouts or dried sugar beet pulp. However, the fiber level for nursing sows must not exceed 4-5% due to negative effects on the utilization of other nutrients. Therefore, it has also been discussed when to start feeding a mixture for nursing sows. The sows have to get used to this feed, so the mixture was mostly started shortly after moving to the farrowing room. However, it has appeared that the fiber level in this mixture is too low for sows in an advanced state of pregnancy, and this causes the metabolism to slow down and other problems as mentioned above. Therefore, some breeders use a mixture for pregnant sows until farrowing and then switch to the next feed. In addition, feed companies now offer the transition mixtures balanced precisely for the farrowing period, which are used as a transition between the mixture for pregnant and nursing sows.

Care for piglets

Obviously, the optimal farrowing would be without complications and take about 3-4 hours. The truth is that small piglets are farrowed more easily due to their size, but their high number increases the duration of the farrowing and increases the risk of colostrum deficiency for the last born piglets. For hyperprolific sows, continuous monitoring should be preferred, and the keeper must be prepared to help both the piglets and the sow immediately if birth complications occur. For piglets, whether small or large, the important factors are drying and supply of colostrum as soon as possible after birth and sufficient heat.

Sufficient intake of colostrum by piglets after the birth is probably the most important factor determining their survival and successful rearing and is particularly important for low birth weight piglets. Colostrum is a source of nutrients, energy for metabolism and heat production, as well as passive immunity that protects piglets until they begin to produce their own antibodies. However, small piglets are less vital and thus less able to make their way to the teats of the sow immediately after birth, while still fighting for colostrum with stronger piglets. Sufficient colostrum intake means that the piglet should receive more than 150 g/kg body weight (*De Vos et al., 2014*). The amount of colostrum received has a significant effect on mortality throughout the pre-weaning period. We can see a decrease in pre-weaning mortality of piglets from 64% to 10% if the amount of colostrum taken exceeds 200 g (*Devillers et al., 2011*). *Diaz et al. (2018)* report that the minimum survival rate is at least 30% body weight. However, in large litters, the amount of colostrum produced may be insufficient. Unfortunately, increased fertility is not accompanied by a proportional increase in the quantity of colostrum, and in fact, we have no strategy that would help us significantly increase the amount of colostrum in the sow. The possibility of how to manage colostrum deficiency is to administer colostrum substitutes. They usually contain a source of energy in the form of fat or lactose, and immunoglobulins and growth factors contained in the milk, or cow's colostrum. However, the effect of these products is not very convincing and does not always allow survival of weak piglets. The best choice is always the colostrum of the sow, specifically the one from the piglets' own mother. Even if we consider moving piglets to another sow, we should allow the piglets to receive their mother's colostrum for at least 6 hours. Small piglets that are unable to accept colostrum by themselves should be assisted by attaching to the teat or by administering colostrum so that they can receive at least a minimum amount.

Consequences of increasing litter size

Increasing litter size, variability, and the number of small piglets results, among other things, in changes in the distribution of pre-weaning mortality. Ten years ago, 70% of piglet mortality occurred on postpartum day one, while today piglet mortality is spread over a longer period of time, and losses occurring on postpartum days 2-7 have almost doubled. A possible cause may be the lack of colostrum in piglets. Perhaps the problem is that the staff identify most of the small piglets as non-viable, which they no longer need to care for, as they are not supposed to survive. In fact, however, large litters of hyperprolific sows have a higher number of small piglets, but these can be fully viable if we provide them with sufficient care. It is therefore important that the staff are able to recognize small piglets from non-viable ones and take care of them accordingly. It helps to weigh piglets during the birth where piglets weighing less than 800 g are determined to be non-viable, small piglets are all piglets weighing more than 800 g and less than 1.2 kg (*Diaz et al, 2018*). If small piglets identified in this manner receive sufficient colostrum and have suitable environmental conditions, including a sufficiently high temperature, their survival chances are relatively high.

With a large variety of litters, we can use split nursing to reduce competition for colostrum and milk and allow small piglets to drink enough. If the number of piglets is higher than the number of functional teats, part of the piglets should be moved to a replacement sow. However, it is advisable not to move the piglets too much and leave them as much as possible without moving with their mother. When moving medium or large piglets, it is advisable to leave them with the mother for at least 6 hours after birth and place them with sows on their 3rd to 5th litter. Small piglets should stay with their mother for at least 12 hours and the most suitable surrogate mother for them will be the sow on her 2nd litter, which does not have too large teats. Cross-fostering, i.e. addition of piglets to other sows, can be done only in the short period after farrowing or for the whole lactation period and the weaning mortality can be reduced by 40%. However, negative effects of movements on growth and thriving of piglets was also reported. In addition, frequent moves promote transmission of viruses (PRRS, circovirus) and are stressful for both piglets and sows.

Timely feeding of piglets

In the next days, we need to ensure, in addition to monitoring the health of the sow and piglets, sufficient heat and dryness in the shelter for the piglets and we can slowly start with the feeding. Its primary objective is for the piglets to get used to the intake of solid food and it is important to adapt the digestive tract of piglets

to the herbal ingredients of the feeding stuffs. Unfortunately, small piglets have poor development and maturation of the small intestine due to limitations of intrauterine development, which impairs digestion of nutrients in the postpartum period. Recently, there has been increasing interest in biologically active substances in the colostrum and milk, which could be used as a supplement for piglets to promote small intestinal growth and maturation. These may include, for example, growth factors (IGF-1, EGF), hormones, or nucleotides. A number of them have been demonstrated to have positive effects during experiments, but their routine use in feeding stuffs is often impossible because they have not been authorized as feed supplements. Nevertheless, the knowledge of all these components of milk that could support the piglet growth is becoming increasingly important because of the percentage of low birth weight piglets.

Milk substitutes and dairy feed mixtures can be used if the sow has little milk. At the end of the lactation period, we can still change the weaning weight of small piglets by split weaning. Large piglets are weaned early so that small piglets can get some extra time with the sow. A minimum of 6 piglets should be allowed to prevent the onset of estrus in the sow.

A number of strategies are available for managing problems of small piglets. Unfortunately, most of them are usually unable to provide the same results under the same economic conditions and with the same amount of care as the breeding of optimally large litters with optimally large piglets. In addition, efforts to get the highest possible fertility in sows pose new problems. For example, increasing the size of the sow's body while reducing the birth weight of the pigs causes small piglets not to reach the top row of teats, although they are sufficiently vital. So the question arises whether to continue this development, or whether it would be advisable to end the pursuit of the highest reproductive performance before we encounter a genetic ceiling associated with many negative effects.

Development of Czech pig production

In the Czech Republic, a total of 1,508,000 pigs are bred, of which 89,000 are sows as at 31 December 2018 according to the Czech Statistical Office. Table 1 shows a number of pigs by weight and purpose of rearing in the Czech Republic. In year-on-year terms this means a decrease of 24 thousand pigs (by 1.6%), especially a decrease of 5 thousand sows (by 5.4%). Despite this reduction in sows during 2018, there was practically identical number of born piglets as in 2017, namely 3.21 million. This confirms the dynamics of improving reproduction indicators, reflecting intense breeding work and increasing selection pressure in pig breeding. The Czech Republic ranks among the developed European countries at the level of reproduction indicators. Compared to 2017, there was a further increase in the

number of born piglets (by 1.3%) and weaned piglets (by 1.1%) for sow and year in 2018 (Table 2).

Table 1. Number of pigs by weight and purpose of rearing in the Czech Republic (year-on-year comparison)

Items	Number as at (head)		Difference (+ , -)	Index (%)
	31. 12. 2017	31. 12. 2018		
Total pigs	1 531 689	1 507 582	-24 107	98,4
Piglets, less than 20 kg of l. w.	476 960	465 705	-11 255	97,6
Young pigs, 20 to <50 kg of l. w.	345 669	320 548	-25 121	92,7
Pigs for fattening (incl. culled sows and boars)	570 938	586 223	15 285	102,7
50 to <80 kg of l. w.	263 701	280 221	16 520	106,3
80 to <110 kg of l. w.	239 729	239 013	-716	99,7
110 kg of l. w. and over	67 508	66 989	-519	99,2
Breeding pigs (50 kg of l.w. and over)	138 122	135 106	-3 016	97,8
Boars	2 072	1 846	-226	89,1
Sows	94 318	89 469	-4 849	94,9
Covered	66 334	63 366	-2 968	95,5
Not covered	27 984	26 103	-1 881	93,3
Gilts	41 732	43 791	2 059	104,9
Covered	19 099	20 587	1 488	107,8
Not covered	22 633	23 204	571	102,5

Source: Czech statistical office

Table 2. Number of total born piglets and weaned piglets per sow and year and piglet mortality in the Czech Republic in 2015 – 2018

Year	Born piglets per sow	Weaned piglets per sow	Piglet mortality (%)
2015	28.6	25.7	10.2
2016	30.1	26.9	10.6
2017	31.2	27.9	10.8
2018	31.6	28.2	11.0

Source: Czech statistical office

The total production of slaughter pigs was 296 300 tons of l.w. in 2017, 304 300 tons of l.w. in 2018 (+ 2.6%). Number and total carcass weight of slaughtered pigs in the Czech Republic is shown in Table 3. In 2018, the share of imports in consumption was 62.2%, the share of exports in production was 27.2%. The largest share (94.0%) in the import of live pigs consisted of piglets, which were imported mainly from Denmark (55%) and Germany (45%) last year. Import of pork to the Czech Republic amounted to 283 300 t of carcass weight in 2018. Germany (34%) is the most important importer of pork in the Czech Republic in

the long term. The share of pork imports from Spain is increasing. Import of pork from third countries remains insignificant.

Table 3. Number and total carcass weight of slaughtered pigs in the Czech Republic - excluding sows and boars

Year	Number of pigs	t carcass weight
2015	2 442 000	220 100
2016	2 364 000	273 200
2017	2 283 000	262 800
2018	2 251 000	258 500

Source: Czech statistical office

The export of live pigs from the Czech Republic in 2018 was carried out by animals for slaughter (63%) and piglets (36%). The main export destinations of live pigs were Slovakia, Hungary and Germany; it was 90% of exports (477.3 thousand pigs). The most important destinations for exports of pork from the Czech Republic were Slovakia (72%) and Germany (14%). The total export was 29 700 t of pork.

Pork consumption was 42.3 kg per capita per year in 2017 and stagnation at the same level is expected in the following period. Self-sufficiency in pork production fell to 51.5% in 2018.

Prices of slaughter pigs in the Czech Republic followed the evolution of prices on the Union market, traditionally mainly prices on the German market. While the price of pigs for slaughter paid to agricultural producers was at the level almost 35 CZK (1.37 €) / kg l.w. in 2017, in the following period it has been decreasing for a long time up to 27.27 CZK (1.07 €) / kg l.w. at the end of 2018. Average price of pigs for slaughter in 2018 markedly fell and virtually corresponds to a historically minimum price in 2010 (Vališ, 2018).

Conclusion

The Czech Republic is one of the countries in which sows performance has increased at the fastest rate of all the monitored EU countries over the past five years, reflecting good-quality breeding work. Nevertheless, we reported a loss in pig production in 2015-2017 due to high production costs. The economic situation in the livestock sector is difficult in the Czech Republic. The Ministry of Agriculture supports the pig farming through national subsidies to improve conditions in pig breeding. With its accession to the European Union the Czech Republic opened the door to the possibility of using finances from EU funds.

Uzgoj svinja u Češkoj – upravljanje prašenjem visoko plodnih krmača

Jaroslava Bělková, Miroslav Rozkot, Eva Václavková

Rezime

Češka se svrstava među razvijene evropske zemlje po nivou pokazatelja u reprodukciji svinja. Uprkos smanjenju broja krmača tokom 2018. godine, broj rođene prasadi je bio gotovo identičan kao i 2017. To potvrđuje dinamiku poboljšanja reproduktivnih pokazatelja, što je rezultat intenzivnog odgajivačkog rada i sve većeg selektivnog pritiska u svinjarstvu. Sve veći broj prasadi u leglu povezan je sa brojnim problemima, uključujući malu težinu na rođenju, slabu ujednačenost legla i povećanu smrtnost prasadi. Zbog toga su potrebne strategije koje će nam pomoći da smanjimo gubitke i optimiziramo odgoj velikih legala. Veći su zahtevi u odgajivanju, tehnologiji i upravljanju odn. menadžmentu i zahtevaju pojačanu negu krmača i prasadi nakon prašenja. Kod današnjim visoko proliferativnih/plodnih krmača preporučivo je koristiti savremene tehnologije koje su prilagođene telesnom okviru, ponašanju i intenzitetu proizvodnje.

Ključne reči: svinjarstvo, menadžment prašenja, velika legla, visoko proliferativne/plodne krmače, privremeno zatvaranje

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FEATURES OF SPERM INJECTION INTO GENITAL TRACTS OF SOWS AND GILTS IN ARTIFICIAL INSEMINATION

Oleksandr Tsereniuk, Oleksandr Akimov, Yuriy Chereuta, Mikola Kosov

Laboratory of Small Animal Husbandry and Horsbreeding, Institute of Animal science of UAAS, Tvarinnikiv Str. 1-A, 61026, Kharkiv, Ukraine

Corresponding author: Oleksandr Tsereniuk, tserenyuk@gmail.com

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Abstract: The purpose of our research was to identify differences in the reproductive system structures of sows and gilts, and on this basis, to study the features of sperm injection into genital tracts of sows and gilts during their artificial insemination. In order to study development of reproductive system of sows and gilts, a slaughter of 5 sows and 5 gilts was carried out at the slaughter point of the basic farm. Separately, the depth of catheter insertion into genital tracts of sows was studied. On the basis of the absolute values obtained, the coefficients of phenotypic consolidation were calculated using Y. Polupan's method (*Polupan, 2002*). The results obtained indicate disproportionate changes in the absolute and linear development of the reproductive system that occur in sows as compared to gilts. The development of uterine horns is not interrelated and doesn't correspond to each other's individual development showing a lack of clear dependence. The difference between sows and gilts is obvious both at the insertion of the catheter, and after the attachment of the vial and the injection of sperm. The results obtained are coordinated with the revealed differences in the weight and linear indices of the reproductive system development of sows and gilts.

Keywords: pigs, artificial insemination, sows, gilts, phenotypic consolidation, insemination catheter

Introduction

The porcine industry is of great importance worldwide, and so any technological innovation in one or more of the associated production areas is of interest for meat production (*García-Vázquez et al., 2019*). At the same time, pig-production is one of the most important directions that allow to increase

significantly the efficiency of pig-breeding using modern methods and technologies, as stated by a number of scientists (*Tsereniuk et al., 2018*). Today, pig production is based on the use of artificial insemination method (AI).

Artificial insemination (AI) of swine is widely practiced in countries with intensive pig production. In Western Europe, more than 90% of the sows have been bred by AI (*Gerrits et al., 2005; Vyt, 2007; Maes et al., 2011*). Use of artificial insemination (AI) for breeding pigs has been instrumental for facilitating global improvements in fertility, genetics, labor, and herd health (*Knox, 2016*).

On farm, AI is the predominant form for commercial sow breeding and relies on manual detection of estrus with sows receiving two cervical or two intrauterine inseminations of the traditional or low sperm doses on each day detected in standing estrus (*Knox, 2016*). There are differences between sows and gilts, as indicated by a number of scientists (*Hernández-Caravaca et al., 2012; Hernández-Caravaca et al., 2017; García-Vázquez et al., 2019*). This aspect is relevant for the further research, since the introduction of new methodological approaches that increase the efficiency of using gilts will greatly simplify their introduction to the main herd.

Accordingly, the aim of our research was to reveal differences in the structure of the reproductive system of the main sows and gilts, and on this basis, to study the particularities of sperm injection into genital tracts of sows and gilts in artificial insemination.

Material and Methods

General principles and approaches for evaluating various groups complied with the requirements accepted in Ukraine, (*Ovsyannikov, 1976*). In order to study development of reproductive system of sows and gilts, a slaughter was carried out at a slaughter point of a basic farm, by 5 sows (average live weight 240 kg \pm 5%) and 5 gilts (average live weight 150 kg \pm 5%). The total weight of the genital organs as a whole, the weight of the uterine horns (left and right), and the weight of the ovaries (left and right) were estimated. Measurements of the linear development of genital organs of sows and gilts were also carried out. The length of the uterine horns (right and left) was determined on the large and the small curvatures, together with development of the ovaries (right and left) according to the parameters of length, width and height. Using absolute indices we have calculated the volume of ovaries, the weight coefficient and ovarian specific weight. In this case, the weight coefficient was calculated for the total weight of the ovaries and separately for the weight of the left and the right ovary.

On the basis of the absolute values obtained, the coefficients of phenotypic consolidation were calculated using Y. Polupan's method (*Polupan, 2002*). The coefficients were determined on the basis of standard deviation (K1) and the coefficient of variability (K2) in relation to the average data for all culled animals (Kc).

Estimation of the catheter insertion depth into the genital dams, in artificial insemination, was carried out for other animals. The sows and gilts were of the same breed. Two-time insemination of sows and gilts was estimated. Standard catheters were used to inseminate the Minitube sows. In each case, 50 measurements were performed. The outer part of the catheter was measured in three positions: at insertion into the sows' genital tracts before attaching the vial of sperm; after attaching the vial; after the outflow of sperm into the sow's reproductive tract. On the basis of the absolute values obtained, the relative part of the injected catheter was measured. The calculation of the phenotypic consolidation coefficients was carried out as in the previous case.

All the data obtained were statistically processed using the computer program Excel in accordance with the methods developed by *Plohinskii (1969)*.

Results and Discussion

With age, in addition to changes in the body structure and the increase in live weight, changes also occur in the absolute, as well as the relative condition of the reproductive system of sows and gilts.

As for the total weight of the reproductive system of the sows, the right and the left uterine horns, there was an increase in absolute values by 23.34-70.11%, compared to the gilts. At the same time, the absolute weight of the left and the right ovaries grew by only 16.11-18.80%. A high level veracity of the difference between the total weight of the reproductive system and the weight of the uterine horns in sows and gilts was established ($p < 0.001$), at the same time no difference in ovarian weight between them was detected.

The highest values of consolidation based on the indicators of the reproductive system development of the gilts are revealed by the weight of the uterine horns (right and left). By the weight of ovaries, the gilts were not consolidated. At the same time, with a minor advantage of the sows over the gilts due to consolidation of the reproductive system weight as a whole and the weight of the uterine horns, there was a significant advantage in consolidation in the weight of the ovaries. Along with this, both the gilts' and the sows' ovarian weights were the least consolidated in the group of indicators of the reproductive system development.

Unlike the absolute weight of the reproductive system, the indicators of its linear development in sows, as compared to the gilts, increased to a lesser extent. Thus, on the large curvature of the left and the right uterine horns, this growth was 3.02% ($p < 0.05$) and 5.64% ($p < 0.01$). At the same time, on the small curvature, the growth of the left horn was higher - 5.65% ($p < 0.001$) compared to 2.98% ($p < 0.01$) of the growth of the left horn, respectively.

The smallest growth of ovarian size was measured by their length - 9.09% on the right and 6.90% on the left; the largest - by their width -13.69% and 15.79% respectively. There were no reliable differences between the ovarian measurements in the sows and the gilts.

Similarly to the absolute parameters of the reproductive system development, its linear parameters revealed a high consolidation in sows.

According to the indicators of large and small curvature of the uterine horns, the sows were consolidated. At the same time, both in the gilts and in the sows, a large consolidation of the linear development of the right uterine horn was revealed on the large curvature and, on the contrary, of the left horn on the small curvature. The least consolidated indicator of the linear development of the gilts' reproductive system was the length, and as for the sows' reproductive system, it was the height of the left ovary.

The weight coefficient of ovaries in the sows increased, as compared to the gilts by 0.043 (for both the ovaries), 0.018 (left) and 0.024 (right). The specific weight, however, on the contrary, decreased - the left ovary by 0.329 and the right one by 0.318, respectively.

After the assessment of the reproductive system development in the gilts and the sows, the depth of catheter insertion was evaluated in artificial insemination of the gilts and the sows (Table 1).

Table 1. Depth of catheter insertion into genital tracts

Indicator	Expression	Gilts	Sows	Difference
Maximal depth of the insemination catheter insertion into genital tracts, cm	$\bar{X} \pm s_{\bar{x}}$	24.32± 0.276	28.06± 0.478***	+3.74
	Cv, %	8.03	12.04	+4.01
Depth of the insemination catheter insertion after the sperm vial attachment, cm	$\bar{X} \pm s_{\bar{x}}$	21.66± 0.269	26.02± 0.395***	+4.36
	Cv, %	8.78	10.74	+1.96
Depth of the insemination catheter insertion after the sperm outflows, cm	$\bar{X} \pm s_{\bar{x}}$	20.20± 0.246	23.46± 0.387***	+3.26
	Cv, %	8.60	11.66	+3.06

The difference between the gilts and the main sows is maintained both at the catheter insertion, and after the attachment of the vial and sperm insertion into

the genital tract. The obtained results are consistent to the detected differences in the weight and linear indices of the genital system development of the sows. At the same time, it should be noted that the established differences between the gilts and the main sows at the maximal depth of the catheter insertion at the level of 15.38% ($p < 0.001$) and after the outflow of the sperm at the level of 16.14% ($p < 0.001$) is less, according to the difference between these two parameters (the maximal depth of the catheter insertion and the depth of the catheter insertion after the outflow of sperm) in the gilts (20.40%) and the sows (19.61%).

On the basis of absolute indices, the share of the catheter insertion in the genital tracts of the gilts and the sows was calculated (Fig. 1).

When the gilts are inseminated using a catheter of an average length, during the attachment of the vial and the insertion of the sperm, at muscle contraction, the catheters are pushed out to an average 8.24% of their total length. A similar process is more pronounced in sows (9.20%). It should also be noted that individual differences between animal groups of the sows and the gilts were more significant in the difference between groups (54.5 and 52.6% at the beginning and at the end of the insemination process in sows, and 33.3 and 35.3% in the gilts respectively).

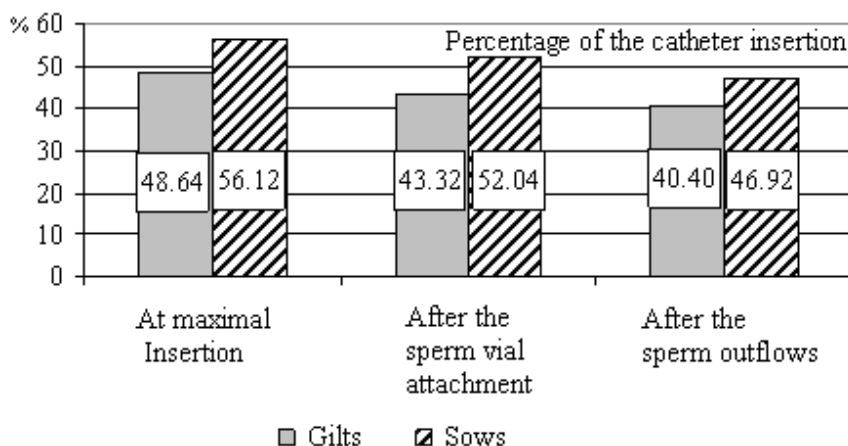


Figure 1. The share of catheter insertion into genital tracts of the gilts and the sows

In general, according to the indicators of the catheter insertion depth into the genital tract, it is also necessary to note the greater consolidation of the gilts (Table 2).

Table 2. Phenotypic consolidation coefficients of the depth of catheter insertion

Indicator	Coefficient	Gilts	Sows
Maximal depth of the insemination catheter insertion into genital tracts, cm	K_1	0.413	-0.015
	K_2	0.368	0.052
Depth of the insemination catheter insertion after the sperm vial attachment, cm	K_1	0.412	0.136
	K_2	0.353	0.208
Depth of the insemination catheter insertion after the sperm outflows, cm	K_1	0.381	0.026
	K_2	0.331	0.094

Conclusion

The results obtained indicate disproportionate changes in the absolute and relative development of the reproductive system that occur in sows as compared to the gilts. The development of uterine horns occurs independently and inappropriately to each other with the absence of a clear dependence on the increase of length linear measurements on the small and the large curvature. The irregularity of ovarian growth with age is due to their length, width and height together with a general decrease in their specific weight. At the same time, the differences between the gilts and the sows are smaller than the differences between the animals within the groups themselves.

Karakteristike ubrizgavanja sperme u genitalni trakt krmača i nazimica u veštačkoj oplodnji

Oleksandr Tsereniuk, Oleksandr Akimov, Yuriy Chereuta, Mikola Kosov

Rezime

Svrha našeg istraživanja bila je da se utvrde razlike u strukturi reproduktivnog sistema krmača i nazimica, i na osnovu toga prouče karakteristike ubrizgavanja sperme u genitalni trakt krmača i nazimica tokom njihove veštačke oplodnje. Da bi se proučio razvoj reproduktivnog sistema krmača i nazimica, zaklano je 5 krmača i 5 nazimica. Odvojeno je proučavana dubina umetanja katetera u genitalni trakt krmača. Na osnovu dobijenih apsolutnih vrednosti izračunati su koeficijenti fenotipske konsolidacije metodom Y. Polupan-a (*Polupan, 2002*). Dobijeni rezultati ukazuju na nesrazmerne promene u apsolutnom i linearnom razvoju reproduktivnog sistema koje se javljaju kod krmača u poređenju sa nazimicama. Razvoj rogova materice nije međusobno povezan i ne odgovara pojedinačnom razvoju koji pokazuje nedostatak jasne zavisnosti. Razlika između krmača i

nazimica očigledna je i nakon umetanja katetera, i nakon pričvršćivanja bočice i ubrizgavanja sperme. Dobijeni rezultati usklađuju se sa otkrivenim razlikama u težini i linearnim indeksima razvoja reproduktivnog sistema krmača i nazimica.

Ključne reči: svinje, veštačka oplodnja, krmače, nazimice, fenotipska konsolidacija, kateter za osemenjavanje

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THE VARIABILITY OF ECONOMICALLY IMPORTANT TRAITS MONITORED IN THE PERFORMANCE TEST OF GILTS UNDER THE INFLUENCE OF FARM, YEAR AND SIRE BREED

Nenad Stojiljković¹, Dragan Radojković², Čedomir Radović¹, Marija Gogić¹, Vladimir Živković¹, Radomir Savić², Aleksandar Stanojković¹

¹Institute for Animal Husbandry, Autoput 16, P.Fax 23, 11080, Belgrade, Serbia

²University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080, Belgrade, Serbia

Corresponding author: Nenad Stojiljković, zake9103@gmail.com

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Abstract: The aim of this study was to determine the influence of sire, sire within the breed, genotype, farm and age on variability of production traits of gilts tested: age at end of the test (AET), life daily gain (LDG), backfat thickness 1 and 2 (BFT1 and BFT2) and back muscle depth (MLD). The production traits of gilts were examined in two pig herds and for three consecutive years. The study included 3605 gilts of 3 genotypes, originating from 82 sire boars. The test included only those boar sires who had 10 or more daughters. In the first herd there were 1233 and in the second 2372 tested gilts. There were 1962 gilts of Landrace (L) breed, 1324 of Large White (LW) and 319 gilts of Duroc (D) breed. Total of 885 animals were tested in the first year, 1145 in the second and 1575 in the third year. Based on the obtained results, it was determined that the genotype had a statistically significant effect ($P < 0.001$) on BFT1, BFT2 and MLD, while the AET and LDG traits were not influenced ($P > 0.05$). Considering the farm as a source of variation of gilts' traits, it was concluded that it had a statistically very highly significant effect on the traits tested ($P < 0.001$). The year of testing of gilts as a source of variation showed a very high statistical effect ($P < 0.001$) on all traits examined. The sire breed statistically highly ($P < 0.001$) led to the variation of the examined traits, which was also the case with the influence of the sires within the breed ($P < 0.001$). The properties BFT1, BFT2 and MLD were highly statistically dependent ($P < 0.001$) on weight at the end of the test.

Keywords: boars, genotype, gilts, performance test

Introduction

The performance test of gilts represents a significant selection measure in pig production. Selection based on performance test results leads to increase in economically important traits (life gain, back fat thickness, MLD depth) by 2-3% per year (*Schinkel, 1999*). The aim of selection on farms is to improve the performance of the herd by increasing the frequency of desirable genes. The relation between hereditary and total variability in a population (herd) is defined by a heritability coefficient. Traits with high and medium heritability can be improved relatively easily by individual selection. Breeding and selection programs involve performing a performance test for these traits by measuring weight, while the thickness of back fat is measured by an ultrasound apparatus (*Đedović, 2015*).

The average backfat thickness values 1 and 2 measured by the PIGLOG 105 apparatus (*Petrović et al., 1999*) were 14.5 mm and 12.5 mm, respectively, these results were obtained at an average body weight at the end of the test of 97.8 kg and at 204 days of age, with an average daily gain of 478 g. In the work of *Michalska et al. (2008)*, the following values are reported in a ten consecutive year study of tested Large White gilts with PIGLOG 105: average age at the end of the test - 180 days, body weight 96.6 kg, LDG 578 g, back fat thicknesses 1 and 2 both 11.4 mm and depth of MLD (back muscle depth) 48.1mm.

Kosovac et al. (2009), by examining the depth of MLD (*Musculus longissimus dorsi*) of the Swedish Landrace genotype have established an average value of 59.25 mm.

At an average body weight of 108 kg over a four-year test period, *Petrović et al. (2002)* have found an average meat content of the tested animals of 57.8%. The influence of breed and genotype on traits tested in performance test has been the subject of research by many authors (*Petrović et al. 1991; Brkić et al. 2001; Kernerová et al. 2006; Nowachowicz et al. 2009; Szyndler-Nędza et al. 2010; Radović et al., 2011*) who are not in concordance/agreement on the influence of these factors on the individual traits examined.

Material and Methods

The production trait of gilts were examined in two pig herds for three consecutive years. The study included 3605 gilts of 3 genotypes, originating from 82 boars sires. The test included only those boar sires with 10 or more daughters. At the end of the test, body weight, fat thickness and MLD depth were measured by an ultrasound apparatus. In the firsther, there were 1233 gilts and in the second 2372 gilts tested. There were 1962 gilts of Landrace (L) breed, 1324 of Large

White (LW) and 319 gilts of Duroc (D) breed. In the first year, 885 gilts were tested, in the second 1145 and in the third 1575 gilts.

Data processing was performed using the appropriate computer program according to the procedure of the Least Squares Method (LSMLMW and MIXMDL-Harvey, 1990) in order to determine the significance ($P < 0.05$) of systematic influences on the age at the end of the test, the life daily gain, the back fat thickness 1 and 2 and muscle depth in performance tested gilts. The models included: sire breed, sires within the breed, farm, age and genotype, as well as the regression effect of weight at the end of the test on the traits examined.

Model 1.

$$Y_{ijkm} = \mu + F_i + G_j + R_k + b_1 (X_i - \bar{X}_i) + \varepsilon_{ijkm}$$

where: Y_{ijkm} = expression of trait in m individual animal, i farm, j test year, of k genotype, μ = general population average, F = farm, G = year of testing, R = animal genotype, b_1 = linear regression effect of body weight at the end of the test, ε = random error, i = subscript for farm ($i = 1, 2$), j = subscript for test year za godinu testiranja ($j = 1, 2, 3$), k = subscript for animal genotype ($k = 1, 2, 3$), m = subscript for offspring.

Model 2.

$$Y_{jkml} = \mu + R_j + O_{kj} + b_1 (X_l - \bar{X}_l) + \varepsilon_{jkml}$$

where: Y_{jkml} = expression of trait in m individual animal, j sire breed, of k sire within j breed, μ = general population average, R = sire breed, O = sires within a breed, b_1 = linear regression effect of body weight at the end of the test, ε = random error, j = subscript for sire breed ($j = 1, 2, 3$), k = subscript for sires within j breed, m = subscript for offspring.

Results and Discussion

All traits tested were reduced to the same weight at the end of the test at 111.00 kg. The average values and standard deviations of the traits examined are shown in Table 1.

Table 1. Average values and variability of traits tested

	Trait	$\bar{x} \pm SD$
AET	Age at the end of test, days	200.19 ± 21.99
LDG	Life daily gain, g	578.10 ± 58.53
BFT1	Back fat thickness 1, mm	9.82 ± 2.31
BFT2	Back fat thickness 2, mm	8.92 ± 1.97
MLD	Back muscle depth, mm	53.41 ± 5.12

Tables 2, 3 and 4 show the $\bar{x} \pm \text{SD}$ values (average and standard deviation) of the examined traits of daughters by sires within the breed and values for the breed.

Table 2. Average values and standard deviations of the examined traits per sire within the Landrace breed

Source of variation		AET ²⁾ , days	LDG, g	BFT1, mm	BFT2, mm	MLD, mm
SB ¹⁾	Sire					
Landrace	33	183.52±10.58	587.36±47.69	8.73±1.10	8.18±1.17	54.58±2.09
	55	228.88±19.49	524.35±51.32	12.24±2.28	10.59±1.84	46.18±3.30
	72	227.47±13.81	499.41±32.84	13.41±1.93	12.06±2.86	47.06±5.38
	75	235.29±11.08	477.14±34.44	11.64±2.34	9.57±2.28	52.86±6.96
	76	224.10±9.11	487.52±36.95	10.93±2.15	9.48±1.72	46.86±5.31
	84	185.61±8.81	596.00±45.62	7.78±1.07	7.11±1.10	55.46±1.57
	Average	212.98±11.67	531.04±38.57	10.80±1.79	9.59±1.75	51.24±4.02

¹⁾ SB-sire breed; ²⁾ AET- Age at the end of test; LDG-life daily gain; BFT1-back fat thickness 1; BFT2-back fat thickness 2; MLD-back muscle depth

Table 2 shows the average values for studied traits of the Landrace boar sires' daughters. The offspring of sire number 33 are the youngest with an average AET of 183.52 days, while the oldest are daughters of sire 75 with age of 235 days. Daughters of sire number 84 show the lowest BFT1 (7.78 mm), while the highest value for this trait was recorded in daughters of sire number 72 (13.41 mm). The difference between the minimum and maximum values for BFT1 is 5.63 mm. The number 76 daughter's daughters are closest to the average for BFT1 within the Landrace breed. As with BFT1, the lowest BFT2 have daughters of sire 84 (7.11 mm) and the highest of sire 72 (12.06 mm), with the difference between the two values being 4.95 mm. The closest to the average for the BFT2 trait are the descendants of sire number 75. The average depth of the MLD is 51.24 mm with a variation from 46.18 to 57.91 mm.

Within the LW sire breed, the oldest are the offspring of sire number 89 (236.45 days), while the youngest the offspring of sire number 57 (182.40 days). Daughters of sire number 22 have the lowest BFT1 (7.96 mm), and the highest daughters of sire number 35 (the difference between being 6.04 mm). The lowest depth of the MLD is recorded in daughters of sire number 77 and the greatest in daughters of sire 19, the difference between them being 11.35 mm. The depth of the back muscle of the daughter of sire number 74 is closest to the average value of this trait for the sire breed LW. When selecting tested gilts as further candidates for selection *Kernerová et al. (2006)* established that for the genotype the Czech LW (CLW) body weight at the end of the test was 127 kg, at the age of 206 days and LDG of 531 g, while for the CLW- sire line genotype, they established an average

mass of 124 kg, at the age of 202 days and an LDG of 511 g. In the present study (Table 3), LDG trait values vary across sires from 473.50 to 612.81 g, but the animals completed the test later (206 days) with a lower body weight (110.98 kg).

Table 3. Average values and standard deviations of the examined traits per sire within the Large White breed

Source of variation		AET ²⁾ , days	LDG, g	BFT1, mm	BFT2, mm	MLD, mm
SB ¹⁾	Sire					
Large White	18	215.67±15.26	522.73±34.22	12.00±2.59	10.47±2.33	50.13±2.61
	19	187.15±10.78	583.92±48.85	8.18±1.11	7.51±1.08	57.91±1.73
	22	187.83±7.34	566.33±31.26	7.96±0.74	7.43±0.77	55.59±3.58
	34	233.25±9.96	473.50±30.44	13.92±2.64	12.08±2.81	48.33±5.05
	35	229.95±8.04	502.42±37.19	14.00±2.19	11.79±2.78	46.89±4.37
	47	223.42±12.61	492.19±34.37	10.87±2.31	9.13±2.03	48.94±6.68
	57	182.40±8.90	612.40±42.64	8.06±0.79	7.48±0.77	57.32±5.03
	63	187.25±11.19	612.81±34.28	8.90±1.00	8.28±0.95	57.74±1.19
	74	186.00±7.14	576.39±27.15	8.50±1.05	8.05±1.03	53.73±3.03
	77	225.17±15.18	475.12±40.96	12.15±2.35	10.71±2.11	46.56±2.82
	89	236.45±12.63	480.77±31.63	11.71±2.12	10.42±1.98	48.74±5.10
	Average	205.77±10.33	542.81±38.41	10.14±1.64	9.05±1.53	52.21±3.60

¹⁾ SB-sire breed; ²⁾ AET- Age at the end of test; LDG-life daily gain; BFT1-back fat thickness 1; BFT2-back fat thickness 2; MLD-back muscle depth

Table 4. Average values and standard deviations of the examined traits per sire within the Duroc breed

Source of variation		AET ²⁾ , days	LDG, g	BFT1, mm	BFT2, mm	MLD, mm
SB ¹⁾	Sire					
Duroc	92	183.90±11.84	603.10±37.69	8.41±1.27	7.88±1.30	56.53±1.50
	25	232.86±8.73	486.36±47.83	15.57±1.27	13.71±2.20	50.43±7.11
	60	230.88±10.84	480.38±47.83	13.44±1.27	11.25±2.32	47.13±6.89
	62	192.50±11.80	606.60±47.83	10.16±1.27	9.47±0.84	54.32±1.10
	Average	207.54±10.53	545.19±33.96	11.44±1.60	10.37±1.49	51.30±4.10

¹⁾ SB-sire breed; ²⁾ AET- Age at the end of test; LDG-life daily gain; BFT1-back fat thickness 1; BFT2-back fat thickness 2; MLD-back muscle depth

Table 4 shows the variations of the examined traits of the daughters of Duroc sires. The lowest value for BFT1 have the daughters of sire number 92 and the highest daughters of sire number 25, with a difference between them of 7.16 mm. As with the BFT1, the lowest BFT2 have gilts from sire 92 and the highest daughters of sire 25 with a 5.38 mm gap between them. Daughters of sire number 60 have the lowest values for back muscle depth, and daughters of sire number 92 have the highest values, with a difference of 9.4 mm between them.

Table 5 shows LSM (Least Squares Average) \pm SE values of the tested properties as effected by factors of farm, year and genotype.

Table 5. LSM (Least Squares Average) \pm SE values of monitored traits in the performance test of gilts with factors farm, age, and genotype

Source of variation		AET ²⁾ , days	LDG, g	BFT1, mm	BFT2, mm	MLD, mm
Farm	1	222.43 \pm 0.45	495.63 \pm 1.32	11.91 \pm 0.06	10.43 \pm 0.06	49.20 \pm 0.15
	2	189.74 \pm 0.36	587.57 \pm 1.06	9.40 \pm 0.05	8.75 \pm 0.05	54.33 \pm 0.12
Year	1	210.43 \pm 0.50	531.44 \pm 1.45	11.08 \pm 0.07	9.91 \pm 0.07	50.14 \pm 0.17
	2	204.75 \pm 0.36	543.98 \pm 1.05	10.57 \pm 0.05	9.56 \pm 0.05	51.24 \pm 0.12
	3	203.07 \pm 0.45	549.37 \pm 1.30	10.31 \pm 0.06	9.30 \pm 0.06	53.92 \pm 0.15
Genotype	1 ¹⁾	206.12 \pm 0.23	541.46 \pm 0.68	10.20 \pm 0.03	9.15 \pm 0.03	52.37 \pm 0.08
	2	205.74 \pm 0.29	542.11 \pm 0.85	10.12 \pm 0.04	9.06 \pm 0.04	52.35 \pm 0.10
	3	206.39 \pm 0.58	541.22 \pm 1.67	11.65 \pm 0.08	10.56 \pm 0.08	50.58 \pm 0.19

¹⁾ 1-SL, 2- LW, 3-D, ²⁾ AET- Age at the end of test; LDG-life daily gain; BFT1-back fat thickness 1; BFT2-back fat thickness 2; MLD-back muscle depth;

When considering the farm as a source of variation of the animals on farm 1 they completed the test with an average of 222.43 days and were by 32.69 days older than animals on farm 2. BFT1 and BFT2 increased on farm 1 by 2.51mm and 1.68 mm, respectively, compared to farm 2. It was found that farm 1 animals had lower back muscle depth by 5.13 mm compared to animals tested on farm 2. Considering gilts genotype, as a source of variation, the Duroc genotype animals were found to have the highest age at the end of the test, whereas the youngest were gilts of Large White breed.

Table 6 shows the significance levels of the effects included in the model on the properties tested in gilts.

Table 6. Statistical significance (significance level) of the factors included in the models on the traits tested

Source of variation (effect)		AET ¹⁾	LDG	BFT1	BFT2	MLD
Model 1	Farm	*** ³⁾	***	***	***	***
	Year	***	***	***	***	***
	Genotype	NS	NS	***	***	***
Model 2	SB ²⁾	***	***	***	***	***
	S:L	***	***	***	***	***
	S:LW	***	***	***	***	***
	S:D	***	***	***	***	***

¹⁾ AET- Age at the end of test; LDG-life daily gain; BFT1-back fat thickness 1; BFT2-back fat thickness 2; MLD-back muscle depth; ²⁾ SB-sire breed; S:LW-sires within Large White breed; S:SL-sires within Landrace breed; S:D-sires within Duroc breed; ³⁾ NS= $P>0.05$; ***= $P<0.001$

Using model 1, it was found that farm and year statistically significantly influenced ($P < 0.01$ and $P < 0.001$) the expression of the tested traits. The gilt genotype statistically significantly ($P < 0.01$ and $P < 0.001$) affects BFT1- back fat thickness 1, BFT2 - back fat thickness 2, MLD - back muscle depth, while the traits AET - age at the end of the test; LDG - lifetime daily gain were unaffected ($P > 0.05$). The effect of year on back fat thickness and muscle depth of the performance tested gilts is consistent with the results obtained by *Nowachowicz et al. (2009)*. In these studies, a significant and highly significant effect of the year on the thickness of P2 back fat tissue (measured behind the last rib 3 cm from the medial plane) was found. Also, *Petrović et al. (1999)* have found that gilt genotype and age had a statistically significant effect on the manifestation of traits in performance tested gilts, which is consistent with the results obtained in this study.

The sire breed and sires within the breed statistically highly significantly ($P < 0.001$) affected all tested traits of performance tested gilts (Model 2). The results obtained are in agreement with the study of *Petrović et al. (1991)* that the production performance traits of the gilts tested varied under the influence of the sires. Also, the statistical significance of the influence of sires within SL and LW breeds was also determined, as confirmed by *Petrović et al. (1999)*.

Table 7 shows the regression effect of the weight at the end of the test on the test traits included in Model 1 and 2 and its significance levels on the tested traits of the gilts.

Table 7. Regression effect of weight at the end of the test on the traits tested and statistical significance (significance level) of the effect on the tested traits included in model 1

Source of variation	BFT1. mm	BFT2. mm	MLD. mm
WET(b)/model 1	0.085	0.078	0.071
F-test/ model 1	*** ²⁾	***	***
WET(b)/model 2	0.081	0.076	0.079
F-test/ model 2	***	***	***

¹⁾ AET- Age at the end of test; LDG-life daily gain; BFT1-back fat thickness 1; BFT2-back fat thickness 2; MLD-back muscle depth; ²⁾ SB-sire breed; WET-weight at the end of the test; ²⁾ NS= $P > 0.05$; *= $P < 0.05$; **= $P < 0.01$; ***= $P < 0.001$

The regression effect of weight at the end of the test on the properties tested using Model 1 shows that with an increase in weight by 1 kg, BFT 1 increases by 0.085 mm and BFT2 by 0.078 mm and the back muscle depth increases by 0.071 mm. The regression effect of the weight at the end of the test on the tested traits (Model 2) shows that with an increase in weight by 1 kg BFT 1 increases by 0.081 mm. a BFT2 by 0.076 mm while the back muscle depth increases by 0.079 mm. The

weight at the end of the test had a statistically significant effect on the properties of BFT1, BFT2 and MLD ($P < 0.001$).

Conclusion

Based on the obtained results, it was determined that the gilt genotype had a statistically significant effect ($P < 0.001$) on BFT1- back fat thickness 1, BFT2 - back fat thickness 2 and MLD - back muscle depth, while AET - age at the end of the test and LDP - life daily gain were not influenced ($P > 0.05$). Considering the farm as a source of variation in gilt traits, it is concluded that the farm had a very statistically significant effect on the traits tested ($P < 0.001$). The year of test as a source of variation showed a high statistical effect ($P < 0.001$) on all traits examined. The sire breed had a statistically significant ($P < 0.001$) effect on the examined gilt traits. Sires within the Landrace, Large, and Duroc breeds statistically highly significantly ($P < 0.001$) affected all tested traits in gilts. The properties BFT1, BFT2 and MLD were highly statistically dependent ($P < 0.001$) on weight at the end of the test. Observing by years, there was an increase in values for LDG, with a decrease in the values for BFT1 and BFT2 and at the same time an increase in the depth of the back muscle. Based on the table 5, animals with fewer days of AET have higher values for the LDG trait compared to older animals, and also have thinner back fat tissue and greater back muscle depth. In the future selection of gilts, it is recommended not to force excessive reduction of the thickness of the back fat, so that the animals would normally reach sexual maturity and successfully bring forth pregnancy and raise the first litter and without difficulty enter into subsequent reproductive cycles. Production on one farm depends directly on the quality of the breeding material. By monitoring and controlling the intensity of the growth in the test, gilts of adequate age and body weight at insemination are obtained. The gilts that do not have the right age, body weight and sufficient back fat thickness at the end of the test, or at the first insemination, do not provide profit, but reduce production efficiency. Low cost is not the goal of any production, which is why recording of different parameters and traits made during breeding of gilts are very important.

Varijabilnost ekonomski važnih osobina praćenih u performans testu nazimica preko efekata farme, godine i rase oca

Nenad Stojilković, Dragan Radojković, Čedomir Radović, Marija Gogić, Vladimir Živković, Radomir Savić, Stanojković Aleksandar

Rezime

Cilj ovog istraživanja je bio da se utvrdi uticaj rase oca, oca unutar rase, genotipa grla. farme i godine na varijabilnost proizvodnih osobina performans testiranih nazimica: uzrast na kraju testa (AET), životni dnevni prirast (LDG), debljina slanine 1 i 2 (DSL1 i DSL2) i dubina lednog mišića (MLD). Proizvodne osobine nazimica ispitivane su u dva zapata svinja u tri uzastopne godine. Istraživanjem je bilo obuhvaćeno 3605 nazimica iz 3 genotipa, koje potiču od 82 nerasta-oca. Ispitivanjem su bili obuhvaćeni samo oni nerasti-očevi koji su imali po 10 i više kćeri. U prvom zapatu je bilo 1233, a u drugom 2372 testiranih nazimica. U okviru rase landras (L) bilo je 1962, veliki jorkšir (LW) 1324 i durok (D) 319 nazimica. U prvoj godini testirano je 885, u drugoj 1145 i u trećoj 1575 grla. Na osnovu dobijenih rezultata utvrđeno je da genotip statistički veoma značajno utiče ($P < 0.001$) na DSL1, DSL2 i MLD, dok na osobine AET i LDG nema uticaj ($P > 0.05$). Uzimajući u obzir farmu kao izvor variranja osobina nazimica, zaključuje se da ona ima statistički vrlo visoko značajan uticaj na ispitivane osobine ($P < 0.001$). Godina testiranja nazimica kao izvor variranja pokazuje vrlo visok statistički uticaj ($P < 0.001$) na sve ispitivane osobine. Rasa oca je statistički visoko značajno ($P < 0.001$) dovođila do variranja ispitivanih osobina, što je bio slučaj i kada se radilo o uticaju očeva unutar rase ($P < 0.001$). Osobine nazimica DSL1, DSL2 i MLD su vrlo visoko statistički zavisile ($P < 0.001$) od mase na kraju testa.

Ključne reči: nerasti, genotip, nazimice, performans test

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THE ROLE OF ANTIOXIDANTS IN BOAR SEMEN PRESERVATION

Elena Cibotaru¹, Grigore Darie², Alisa Pirlog¹, Doina Plesca²

¹The State Agrarian University of Moldova

²The Scientific and Practical Institute of Biotechnologies in Animal Husbandry and Veterinary Medicine

Corresponding author: lenuta_mar@yahoo.com, darie@mail.ru

Original scientific paper

Abstract: The aim of the research was to test the biologically active substance obtained by the Institute of Microbiology of the Science Academy from Moldova. It was introduced in the basic medium as an additional component for boar semen dilution. The obtained experimental data showed that the introduction of 7% of the biologically active substance in the basic medium had positive results in the preservation of the semen material quality at 16-18° C. After 96 hours of conservation at 16-18° C the sperm motility of the spermatozoa was 54,3%.

For the first time, it has been demonstrated that the capacity of the biologically active substance has demonstrated specific and non-specific characteristics to increase the preserved sperm resistance at 16-18° C. There was studied the average speed of the sperm with velocity average path (VAP), the average speed of the sperm with straight linear velocity (VSL) and curvilinear velocity (VCL) during the preservation at the hypothermic temperatures. The proposed medium for the dilution and preservation of the boar sperm at 16-18° C contains: glucose, sodium citrate, helaton, ammonium sulphate and the biologically active substance. The application of this medium allows to use more efficiently valuable reproduction characteristics of boars in the artificial insemination system.

Keywords: boar, sperm, medium, speed of movement, preservation, hypothermic temperature.

Introduction

One of the important technological issues in the pig industry is to improve the reproduction of the herd when using artificial insemination. Pork production enterprises use fresh, or transported in a chilled state, semen of high-value boars

instead of the traditional delivery of boars in order to eliminate inbreeding and to improve breeding.

One of the urgent problems of the animal husbandry science is to increase the efficiency of herd reproduction by maximizing the use of high-value producers, which is of great importance for ensuring further progress in animal husbandry. The use of artificial insemination is an important direction of improving the breeding and productive qualities of animals. One of the determining factors for the effectiveness of artificial insemination is the improvement of sperm storing methods (*Epshina, 2011*).

Considering the peculiarities of boar spermatozoa, which react differently to thermodynamic processes and, consequently, to the formation of protective reactions and adaptive properties during storage outside the body, this is an urgent problem (*Narizhny, 2001*).

In this regard, it seems promising to develop more efficient medium for the short-term storage of boar semen using biological active substances and other cry protectants.

The speed of sperm movement is a characteristic feature of all animal species. Its evaluation is done by different methods. It was established that sperm motility is normal if the spermatozoa pass the field of view of the counting chamber 0.06 ml in 0.9 seconds. The deviation from the normal forms of sperm movement often occurs; it depends on the influence of various factors of functional or morphological nature (*Zăhan, 2017*). As the author notes, the assessment of sperm motility by computer programs is considered more modern and more effective than the traditional methods for assessing the sperm quality. A computer assessment of sperm motility was used to study boar semen (*Holt et.al. 1977*), rams (*Martiner and Maswell, 1999*), stallions.

The determination of the quality of diluents by the method of a test medium for motility and survival of spermatozoa outside the body is important. However, the most important biological properties of sperm, and namely the speed of sperm advance, remain relevant. That is why, in a detailed study of the quality of diluents, along with a bio control method, more functional methods should be applied to test the action of the tested diluents on the biological properties of spermatozoa, such as their speed of movement, which seems to correlate with fertilizing ability.

Material and Methods

In our research, we took as a basis, the production medium GCChS - glucose, citrate, chelate, sulphate medium for dilution of sperm from boars.

It was necessary to identify the optimal concentration of the biologically active substance preparation introduced as an additional component into the basic

medium and prolonged exposure of the sperm at 16-18⁰ C, as well as to determine the physical state of the germ cells under these storage conditions.

The study was conducted on the sperm of the boars of Landrace breed. After an eye evaluation, each ejaculate was divided into 11 parts and diluted with production medium - GCChS containing from 1 to 10 % of the biologically active substance preparation developed by the Institute of Microbiology and Biotechnology of the Academy of Sciences of the Republic of Moldova. The diluted sperm was kept at a temperature of 16-18⁰ C. The sperm motility was determined every 24 hours.

These indicators were determined using the CEROS program.

VAP is a measurement of a common path taken by spermatozoa for a certain period of time. VSL is a straightforward path traveled by spermatozoa from one to the other for a certain observation period. VCL - the total path traveled by the sperm head for the period of observation.

Results and Discussion

The experiment tested the effect of the biologically active substance preparation on the biological properties of spermatozoa during their storage at 16-18⁰ C.

The data on the study of the effect of the biologically active substance preparations an additional component in the composition of the basic medium are presented in Table 1.

Table 1. Spermatozoa motility depending on the preparation concentration, %

Concentration of the biologically active substance, %	1 day		2 days		3 days		4 days	
	Motility, %	Straight linear velocity, %	Motility, %	Straight linear velocity, %	Motility, %	Straight linear velocity, %	Motility, %	Straight linear velocity, %
1	84.5±5.5	42.8±7.9	75.0±2.8	29.8± 2.4	73.3±10.3	28.8±7.3	45.3±18.5	14.8±6.7
2	87.0±5.6	45.0±6.1	79.3±8.7	36.0±8.9	66.0±9.0	22.5±6.1	49.8±17.6	16.5±6.0
3	85.8±5.1	44.3±6.4	81.0±6.1	33.5±5.8	71.3±9.7	27.3±7.1	33.3±20.0	10.5±6.5
4	83.8±6.0	41.5±4.9	78.8±7.1	32.3±6.2	70.0±9.0	24.5±4.3	39.0±19.2	14.8±8.2
5	89.3±3.3	45.5±3.0	78.8±8.8	35.8±6.8	73.5±8.9	29.8±6.2	44.3±20.6	16.0±8.4
6	87.3±1.7	43.0±1.9	73.0±7.6	34.5±4.9	73.3±9.7	31.5±6.0	52.5±18.6	20.0±7.8
7	85.3±3.2	47.0±4.6	81.5±3.0	37.5±1.2	77.0±9.1	32.0±6.4	54.3±15.4	18.3±7.4
8	83.0±3.5	39.8±5.5	78.3±6.8	38.8±4.6	62.5±10.7	21.3±7.1	23.8±18.5	9.8±7.3
9	92.3±1.7	47.8±1.7	78.0±9.7	27.8±6.4	73.3±8.9	31.3±6.2	45.3±10.4	12.3±4.8
10	86.8±2.4	41.8±4.4	78.3±8.5	33.3±7.7	64.5±8.9	25.3±5.6	41.3±16.3	14.3±6.3

The research results show that biologically active substance preparation introduced into the basic medium (GCChS) is not toxic to spermatozoa within the studied concentration. Sperm motility after dilution in all the studied media corresponded

on average from 83.0% when 8% of the biologically active substance was introduced into the medium and 92.3% when 9% of the biologically active substance was added to the medium.

During the storage of sperm at 16-18⁰ C sperm motility has changed. The best motility results were obtained when 7% of the biologically active substance preparation was administered to the basic medium. After 96 hours of semen storage, the motility was 54.3%. The same changes occurred in the number of sperm with straight linear velocity. If, immediately after dilution, the number of sperm cells with straight linear velocity was on average 40% in all the tested media, then during the storage process of the diluted sperm this indicator decreased. The best results were obtained when 7% of the biologically active substance preparation was introduced into the basic medium; after 96 hours storage, the amount of spermatozoa with straight linear velocity was 18.3%.

When measuring the speed of sperm movement, the CEROS program determines three indicators: average speed — VAP is the average speed passed by the spermatozoa over a certain period of time. VAP measurement data are presented in Table 2.

Table 2. Velocity average path of the boar semen (VAP) $\mu\text{m/s}$

Concentration of the biologically active substance, %	24 h	48 h	72 h	96 h
1	98.1 \pm 1.5	85.1 \pm 7.6	72.1 \pm 7.6	47.7 \pm 16.7
2	108.7 \pm 9.5	89.8 \pm 9.2	74.4 \pm 10.0	54.3 \pm 18.5
3	95.6 \pm 7.1	83.2 \pm 12.7	79.3 \pm 8.8	29.3 \pm 17.2
4	90.0 \pm 3.2	81.7 \pm 8.2	77.8 \pm 11.7	45.5 \pm 17.2
5	104.6 \pm 9.1	76.1 \pm 9.0	77.2 \pm 9.7	50.2 \pm 17.6
6	98.7 \pm 1.6	82.3 \pm 4.1	82.6 \pm 7.2	49.0 \pm 16.9
7	96.8 \pm 5.4	80.0 \pm 6.0	85.4 \pm 12.6	65.2 \pm 11.9
8	94.8 \pm 5.8	85.4 \pm 6.1	67.9 \pm 10.6	34.1 \pm 19.7
9	99.0 \pm 6.6	76.3 \pm 9.1	85.3 \pm 8.2	55.2 \pm 12.0
10	97.1 \pm 2.6	79.4 \pm 10.8	70.4 \pm 7.4	47.9 \pm 16.1

The data presented in Table 2 show that the total path traveled by the spermatozoa for a certain period of time immediately after the dilution of the sperm in all the tested media has remained almost unchanged. However, in the research process the quality of sperm has changed. The best results were obtained when 7% of the biologically active substance preparation was introduced into the main diluent. The total path passed by the sperm after 96 hours of the sperm storage was 65.2 \pm 11.9 $\mu\text{m} / \text{s}$.

When the speed of sperm movement is measured by the CEROS program, the straight-line path passed by sperm from one point to another is measured for a certain period of observation. This is the lowest digital value compared to the other measurements carried out by the CEROS program.

The data are presented in table 3.

Table 3. Straight linear velocity of boar spermatozoa (VSL) $\mu\text{m/s}$

Concentration of the biologically active substance, %	24 h	48 h	72 h	96 h
1	51.4 \pm 3.7	44.6 \pm 6.5	36.6 \pm 3.8	33.5 \pm 15.3
2	59.1 \pm 6.1	48.0 \pm 5.7	37.8 \pm 5.7	27.9 \pm 9.5
3	50.4 \pm 6.0	44.5 \pm 5.1	39.9 \pm 5.5	15.7 \pm 9.3
4	47.6 \pm 2.9	43.0 \pm 4.2	37.6 \pm 4.0	25.0 \pm 9.1
5	56.2 \pm 4.0	41.7 \pm 4.1	40.9 \pm 4.9	25.6 \pm 9.3
6	53.7 \pm 0.5	44.4 \pm 1.7	41.3 \pm 3.8	26.4 \pm 9.1
7	52.5 \pm 1.9	44.5 \pm 2.3	44.7 \pm 6.2	33.8 \pm 5.5
8	52.4 \pm 2.9	48.3 \pm 2.7	36.0 \pm 6.3	18.4 \pm 10.6
9	54.5 \pm 2.6	41.8 \pm 3.3	43.2 \pm 2.8	29.3 \pm 3.7
10	51.6 \pm 2.8	42.8 \pm 5.5	36.8 \pm 3.4	25.4 \pm 8.7

Measuring the straight path traveled by the sperm from one point to another for a certain time we have established that the best results were obtained when 7% of the biologically active substance preparation was introduced into the basic medium. After 96 hours of sperm storage at 16-18⁰ C, the speed of spermatozoa movement was 33.8 \pm 5.5 $\mu\text{m/s}$.

The data on the study of the total path traveled by the sperm head VCL for the observation period are presented in Table 4.

Table 4. Curvilinear velocity of boar spermatozoa (VCL) $\mu\text{m/s}$

concentration of the biologically active substance, %	24 h	48 h	72 h	96 h
1	186.1 \pm 8.1	150.1 \pm 10.1	115.4 \pm 32.1	82.7 \pm 28.5
2	191.2 \pm 10.5	159.3 \pm 15.3	131.7 \pm 17.1	96.7 \pm 32.4
3	178.8 \pm 10.9	147.2 \pm 23.1	139.8 \pm 14.4	52.3 \pm 30.2
4	167.2 \pm 10.9	144.2 \pm 15.3	143.8 \pm 18.8	84.9 \pm 30.3
5	191.3 \pm 12.6	138.1 \pm 14.2	139.3 \pm 15.5	87.1 \pm 30.7
6	179.7 \pm 7.1	148.0 \pm 12.3	155.9 \pm 14.9	85.8 \pm 28.9
7	185.9 \pm 13.8	144.6 \pm 12.6	155.6 \pm 21.0	115.2 \pm 22.2
8	176.8 \pm 8.6	152.8 \pm 10.5	123.2 \pm 18.3	57.0 \pm 32.9
9	187.4 \pm 10.9	134.9 \pm 18.7	157.6 \pm 15.3	97.7 \pm 20.3
10	179.2 \pm 6.6	145.8 \pm 20.5	131.1 \pm 12.3	83.6 \pm 28.1

The obtained data show that this is the largest digital indicator in comparison with other measurements. It was also found that the best results were obtained when 7% of the biologically active substance preparation was introduced into the primary diluent. The total path traveled by spermatozoa in this variant of the experiment was $115.2 \pm 22.2 \mu\text{m/s}$.

Despite the fact that the CEROS program conducts a lot of researches to determine the quality of sperm, recently in the specialty literature there have been disputes whether CEROS indicators can be used to predict the fertilizing ability of the sperm.

Conclusions

For the first time, the possibility of using biologically active substance preparation, a substance with specific and nonspecific actions to increase the biological ability of spermatozoa, introduced as an additional component in the composition of GCChS - medium, has been studied.

Uloga antioksidanata u očuvanju sperme nerastova

Elena Cibotaru, Grigore Darie, Alisa Pirlog, Doina Plesca

Rezime

Cilj istraživanja bio je testiranje biološki aktivne supstance koju je dobio Institut za mikrobiologiju Akademije nauka iz Moldavije. Unesen je u osnovni medijum kao dodatna komponenta za razblaživanje sperme nerasta. Dobijeni eksperimentalni podaci pokazali su da je unošenje 7% biološki aktivne supstance u osnovni medijum imalo pozitivne rezultate u očuvanju kvaliteta semenskog materijala na 16-18°C. Posle 96 sati čuvanja na 16-18°C pokretnost spermatozoida bila je 54,3%.

Prvi put je zabeleženo da je sposobnost biološki aktivne supstance pokazala specifične i nespecifične karakteristike za povećanje očuvane otpornosti sperme na 16-18°C. Ispitana je prosečna brzina sperme sa prosečnom brzinom putanja (VAP), prosečna brzina sperme sa ravnom linearnom brzinom (VSL) i krivolinearnom brzinom (VCL) tokom konzerviranja na hipotermičkim temperaturama. Predloženi medijum za razređivanje i očuvanje sperme nerastova na 16-18°C sadrži: glukozu, natrijum citrat, helaton, amonijum sulfat i biološki aktivnu supstancu. Primena ovog medijuma omogućava da se efikasnije koriste reproduktivne karakteristike nerastova u sistemu veštačke oplodnje.

Ključne reči: nerast, sperma, medijum, brzina kretanja, očuvanje, hipotermička temperatura.

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INSECTS-A NEW BRANCH OF ANIMAL HUSBANDRY?

Ksenija Nešić, Marija Pavlović, Vladimir Radosavljević

Institute of Veterinary Medicine of Serbia, Autoput 3, 11070 Belgrade, Serbia

Corresponding author: Ksenija Nešić, ksenija.nesic@gmail.com

Review paper

Abstract: Growing world population will have additional two billion people by 2050. A rise in world hunger has already been evident, while food crises are also caused by climate variability and extremes. Future demands for feed and food production will maximise the pressure on scarce agricultural resources. Global need for meat, milk, eggs and consequently for animal feed will increase. Particularly protein supply will have to elevate significantly. Agriculture sector will be forced to compete for land and water with expanding urban settlements and industrial zones, while offering ecologically friendly products and will have to maintain renewable and sustainable systems. All of this presents a challenge for the scientific community to find solutions. Therefore, the insects have become extremely acceptable possibility, even in parts of the world where traditionally they have never had a desirable status. Since the mid-2017 seven species have been allowed to be reared and integrated in aquaculture feed in EU, with further expansion expected. Thus, the Western world is paying more attention to the use, and also to the cultivation of these animals. The paper presents the literature data in the field of farming insects that can serve to develop this practice in the European conditions in future.

Key words: entomophagy, farming insects, feed, food

Introduction

Entomology (from Ancient Greek έντομον (entomon), meaning “insect” and λογία (logia), meaning “study of”) is the study of insects, a branch of zoology. Class Insecta belongs to the kingdom *Animalia*, so they are animals. Although they are often considered a nuisance to human beings and mere pests for crops and animals, insects are crucial components of many ecosystems, where they perform very important functions. They aerate the soil, pollinate blossoms and control insect and plant pests. Many insects, especially beetles, are scavengers, feeding on dead corpses and plants, thereby recycling nutrients back into the soil. As decomposers, insects help create top nutrient-rich layers of soil that help plants

grow and, thus, participate in waste bioconversion. Burrowing bugs, such as ants and beetles, dig tunnels that provide channels for water, benefiting plants. Finally, all insects fertilise the soil with the nutrients from their droppings. Some species produce useful substances, such as honey, wax, lacquer or silk, while some have also been used in medicine (Nesic and Zagon, 2019).

It is estimated that insects form part of the traditional diets of at least 2 billion people and more than 1,900 species have reportedly been used as food (FAO, 2013). Entomophagy (from the Greek terms έντομος (entomos) – “insect” and φάγειν (faguein) – “to eat”) has had a great importance for almost all humans’ diet throughout history. They are commonly a food source in Asia, Africa, Central America and South America. In European countries, however, people usually perceive eating insects with disgust and associate it with rather exotic ethnical habits. Starting in 2018, Regulation (EU) No. 2015/2283 entered into force, laying down provisions for the approval of novel foods in Europe (European Union, 2015), including insects.

On the other hand, insects are eaten by many amphibians, reptiles, birds and mammals, making their roles in food chains irreplaceable. The search for alternative and sustainable proteins is an issue of major importance that requires viable solutions in the short term, making them an increasingly attractive feed option. Therefore, in mid-2017, the European Commission adopted the amendment EU Regulation No. 2017/893 (European Union, 2017), allowing seven species to be reared and used in feeding aquaculture. This list of authorised insects includes: black soldier fly (*Hermetia illucens*), common housefly (*Musca domestica*), yellow mealworm (*Tenebrio molitor*), lesser mealworm (*Alphitobius diaperinus*), house cricket (*Acheta domesticus*), banded cricket (*Gryllodes sigillatus*) and field cricket (*Gryllus assimilis*).

Farming purpose

Insects are mainly produced as animal feed or human food, so they should compare favorably to conventional protein products. Content of protein is high, as it is the case with the larvae of black soldier fly (*Hermetia illucens*) and house fly (*Musca domestica*) which is up to 63% (Makkar et al, 2014). They possess significant levels of key amino acids (e.g. lysine, tryptophan) when compared to most crop plants. A recent study comparing nutritive characteristics of a range of insects has shown that the amino acid profile of dipteran insects is superior to soybean meal and more similar to fishmeal (Barroso et al., 2014).

Next main component of insects after protein is fat (Doberman et al., 2017). The unsaturated fatty acid profile is similar to that of poultry and white fish but contains more polyunsaturated fatty acids (PUFAs) than either poultry or red

meat (*Rumpold and Schluter, 2013*). Carbohydrates in insects are formed mainly by chitin. As it is reviewed by *Mrcek et al. (2014)* the carbohydrate content of edible insects ranged from 6.71% in sting bug to 15.98%. Mean estimates show energy levels to be around 400–500 kcal per 100 g of dry matter, making it comparable with other protein sources (*Payne, 2016*).

Studies dealing with the vitamin content in insects are insufficient, yet it is known that edible insects contain mainly carotene and vitamins B1, B2, B6, D, E, K and C. Vitamin A has been found to range from 3 to 273 µg/100 g dry matter across insect species (*Christensen, 2006*). Analysis of mineral elements showed (*Rumpold and Schluter, 2013*) that edible insects are rich in these nutrients as well. Nutritional value of insects varies depending on the species, diets and life stages.

It is technically feasible to mass-produce insects for human consumption using industrial methods. Whether automation is economically interesting depends largely on labour costs. Furthermore, automation has the advantages of increased product performance and consistency, reduction in microbial contamination by personnel, and increased space utilization. Insects such as the silkworm (*Bombyx mori*), the termite *Macrotermes subhyalinus* and the drugstore beetle (*Stegobium paniceum*) have been even considered for space-based agricultural systems because they can recycle waste material (*Katyama et al., 2008*). A drugstore beetle growth reactor large enough to provide 100 people with animal protein occupies only 40 m³.

Farming systems

Most edible insects in the world are harvested from the wild, which has a comparative advantage over gathering from crops as they are free from pesticides (*van Huis, 2013*). However, overexploitation has led to the disappearance of some insect species, like in Africa, or problems with deforestation as harvesting of e.g. caterpillars is more easily done by cutting down the trees. In Mexico, 14 edible insect species were documented as threatened due to overexploitation or ecosystem degradation (*Ramos-Elorduy, 2006*). Therefore, methods conducive to insect survival and reproduction should also be developed.

Domesticated species since long time ago are silkworms and honey bees because of their by-products, although in both cases the insects themselves are also eaten. Recent examples of edible insects being commercially farmed for human consumption include the house cricket (*Acheta domesticus*), the palm weevil (*Rhynchophorus ferrugineus*), the giant water bug (*Lethocerus indicus*), especially in Asian countries.

However, when promoting insects as food and feed in the United States and Europe, procedures for large-scale rearing need to be developed. This is a challenge for industries specialized in the mass rearing of insects for biocontrol, sterile insect technique and pet feed. The major issues in mass rearing are quality, reliability and cost-effectiveness. In addition, pathogens (such as the *A. domesticus* densovirus) can constitute a serious problem in commercial rearing. Two examples from literature can serve as a possible model.

Farming crickets

Hanboonsong et al. (2013) described farming technology of house crickets in Thailand which are mainly used for human consumption. It started in 1998 and the technology was developed by entomologists at Khon Kaen University. It was then disseminated to interested farmers countrywide through training courses. It was also introduced at primary schools as small-scale practice which also produced additional protein for the school lunch programme. The main cultivated species in Thailand today is *Acheta domesticus* which is imported from temperate regions in Europe and the United States. The period of development from egg to adult is 45 to 60 days. As published by *Korkka (2016)*, the house cricket, *Acheta domesticus*, is easy to farm, apart from their escape attempts, and can produce from 6 to 7 generations per year. It is omnivorous and can eat a large range of organic materials. Production is feasible at temperatures higher than 20°C, the ideal temperature being 28-30°C. 2000 insects can be bred on 1 m².

Four types of breeding containers are used in cricket farms (*Hanboonsong et al., 2013*). Concrete cylinders are approximately 80 cm in diameter and 50 cm high. They can produce around 2 to 4 kilograms of crickets. One person can easily take care of 20 to 30 units. Concrete block pens are rectangular and interconnected. The sizes vary depending on space availability; 1.2 x 2.4 x 0.6 metres is common. The number of blocks varies from 5 to 100 per farm. Each pen can produce 25 to 30 kilograms of crickets. The rectangular shape is an efficient way of using space, but there is risk of disease outbreak or overheating as the cricket population is always crowded. Third type of boxes resemble the concrete blocks and are usually made from plywood or gypsum board. Size is about 1.2 x 2.4 x 0.5 metres and can produce 20 to 30 kilograms of crickets. The bottom section is elevated off the ground by four 15-20 cm high legs, so the unit is movable. It is easy to clean and does not build up as much heat as the concrete block pens. However, the boxes are less durable than the concrete blocks. Plastic drawers square and around 0.8 x 1.8 x 0.3 metres in size are also in use. A set of three to four drawers is stacked on a shelf and can produce 6 to 8 kilograms of crickets. They need very little space and are suitable for small and medium size

farms. They are easy to look after and can be moved. However, they are not durable and are prone to overheating.

Containers could be covered with mosquito nets to keep crickets in and predators out. The bedding is often made from a layer of rice husks but some breeders do not use any material. Cardboard egg cartons can be used. As soon as the male crickets stridulate, bowls containing a mixture of husk and sand are placed in the breeding enclosure in which females can lay eggs (within 24 hours). Egg laying duration is seven to fourteen days. The bowls are moved daily to another breeding tank for incubation and hatching, usually after about seven to ten days, in a stable temperature. This reproduction cycle can be repeated one to three times for each generation. After the mating period occurs (usually between 40 to 45 days of the life cycle) the crickets can be collected. In Thailand eggs to start a cricket farm can be bought from other cricket farmers, or by catching adult male and female crickets from the wild and keeping them in a closed container with bowls containing a mixture of husk and sand for egg laying purposes. The number of egg bowls needed to begin a cricket farm will depend on how many pens and the size of pens involved; for example 35 egg bowls for one concrete breeding container of 2.2 x 4.8 x 0.6 metres. One egg bowl can produce 3 kilograms of adult crickets.

In cricket farming commercial high protein animal feed, particularly chicken feed 14 or 21 % protein, is widely used. The 21 % protein feed is used for feeding crickets after hatching until they are 20 days old. Subsequently they are fed with mixed 14 and 21 % protein feed until harvesting at 45 days old. A few days before harvesting, the high protein feed is replaced with vegetables such as pumpkins, cassava leaves, morning glory leaves and watermelons to improve taste and to reduce use of the more expensive protein feed. The wholesale buyers wash and boil the crickets and then package them in 5 kilogram packs.

Constraints and threats to cricket farming is a high price of high protein feed, which makes approximately half of the production cost. Research is needed to develop a special feed formula for crickets. Currently, disease risk is almost non-existent. There have been a few cases of crickets dying without any disease symptoms or pathogens. It is suspected that overcrowding or contamination by fungi in the feed may have been the cause. In-breeding is another risk which is expressed in less active and slow-maturing crickets and notable after three generations, so outsourcing breeding stock and eggs should be the best practice.

Farming mealworms

Farming of mealworms is rather simple. *Tenebrio molitor* lifecycle is 3 months during which they go through 4 stages: from eggs to larvae, to pupae and then beetle. The reproduction of mealworms is very high: one female *T. molitor*

produces 160 eggs in their lifetime (*Oonicx and Boer, 2012*). It is done in containers of various sizes, in a climate controlled rearing station. The mealworms with their substrate are held in cages until their size is optimal for collection. They are then put through a sieve which separates the insects of different sizes: small worms which go back to the rearing station, optimal sized worms which are cleaned and are ready to be used, then breeder animals which are at pupae stage (cocoons) are separated into a different cage. After hatching the breeder beetles produce eggs that are separated when they start to appear and put into the rearing chamber. Whenever there are dead breeder animals in the breeder chamber they are removed. The cages used in the rearing chamber are cleaned every 2-3 months to keep the rearing station clean and prevent possible complications in the process. The feed for the worms is constantly kept at 4-6cm height. For feed mealworms can use a wide variety of material, commonly carrots and cereals. Mealworms require a temperature of over 24°C and often 28–30°C is the temperature used. The relative humidity in which mealworms perform well is 60%. Harvesting of mealworms is done before the larvae reach pupal stage because at that point they start losing their bodyweight. For efficient production of mealworms the larvae should be collected when they are about 100-110 mg in bodyweight for maximum efficiency, this bodyweight is achieved at 8-10 weeks of age. Then the insects are starved for 24 hours so that they will excrete all their faeces before putting them in a freezer, which finally kills them without unnecessary pain (*Korkka, 2016*).

Feeding insects

Besides already mentioned chicken feed, also different plants, vegetables and waste streams could be used as feed for insects. Miech (2018) examined use of different weeds and agricultural by-products (rice bran, cassava tops, water spinach, spent grain and mungbean sprout residues) as feed for Cambodian field crickets (*Teleogryllus testaceus*) and the value of crickets as feed for monogastric animals. The obtained results showed that field crickets could be reared using simple means, with *e.g.* cassava tops and *C. rutidosperma* (purple cleome) as feed.

As an increased demand for meat will cause a more than proportional demand for grain and high-protein feeds, feed conversion ratios (FCRs) become even more important. FCRs vary widely depending on the class of animal and the production practices used to produce the meat. However, from some long-term statistics, the following FCRs were given by *Smil (2002a)*: 2.5 for chicken, 5 for pork and 10 for beef. There are few studies on FCRs for edible insects. Different FCR values are given for *Acheta domesticus*: 0.9–1.1 depending on the diet composition (*Nakagaki and deFoliart, 1991*) and 1.7 for fresh weight (*Collavo et*

al., 2005). The difference is probably due to longer feeding and later harvesting in the second study (21 days versus 45 days).

The proportion of edible weight differs considerably between conventional livestock and insects. The percentage of edible weight for chicken (*Flachowsky, 2002*) and pork (both 55% of live weight) is higher than that for beef (40%) (*Smil, 2002b*). Crickets in the last nymphal stage can be eaten whole, but when eaten as a snack, some prefer that legs (17% of total weight) be removed, and because the chitinous exoskeleton (3%) is indigestible, the percentage edible weight amounts to 80% (*Nakagaki and de Foliart, 1991*). Using these data, the FCR of edible weight can be calculated, showing crickets to be twice as efficient as chickens, 4 times more efficient than pigs and 12 times more than cattle (Table 1).

Table 1. Efficiencies of production of conventional meat and crickets

	Cricket	Poultry	Pork	Beef
Feed conversion ratio (kg feed:kg live weight)	1.7	2.5	5	10
Edible portion (%)	80	55	55	40
Feed (kg:kg edible weight)	2.1	4.5	9.1	25

Calculations can also be made for protein efficiency. That would be helpful if the protein content was very different for livestock and crickets, but it is not. Poultry, pork and beef show values of 200, 150 and 190 g protein per kilogram edible weight, respectively (*Flachowsky, 2002*), whereas for cricket nymphs and adults, these figures are 154 and 205 g, respectively (*Finke, 2002*). It is most likely that crickets convert feed more efficiently to body mass than do conventional livestock because insects are poikilothermic and their growth stages do not invest metabolic energy in maintaining a constant body temperature above ambient values. Other insect species are therefore likely to show similar efficiencies (*van Huis, 2013*).

Conclusion

Based on recent data, insects represent a promising feed and food protein source, but for greater European implementation more research needs to be done. It has to be examined how the nutritional value of insects can be managed systematically, establish clear processing and storage methodologies, define rearing practices and implement regulations and adequate laboratory control with regard to food and feed safety. The final decision, as for any novel ingredient, is up to the consumer, accompanied by thorough risk assessment and correct labelling.

Insekti - nova grana stočarstva?

Ksenija Nešić, Marija Pavlović, Vladimir Radosavljević

Rezime

Do 2050. godine rastuća svetska populacija imaće još dve milijarde ljudi. Porast gladi u svetu je već evidentna, dok su krizne situacije u snabdevanju hranom uzrokovane i klimatskom varijabilnošću i ekstremnim vremenskim prilikama. Budući zahtevi za proizvodnju hrane i hrane za životinje maksimalno će povećati pritisak na oskudne poljoprivredne resurse. Globalna potreba za mesom, mlekom, jajima i posledično za hranom za životinje će rasti. Naročito će se snabdevanje proteinima značajno poljuljati. Sektor poljoprivrede će biti prisiljen da se utrkuje za zemljište i vodu sa širenjem urbanih naselja i industrijskih zona, a istovremeno će morati da nudi ekološke proizvode i da održava obnovljive sisteme. Sve ovo predstavlja izazov za naučnu zajednicu da pronađe rešenja. Stoga su insekti postali izuzetno prihvatljiva mogućnost, čak i u delovima sveta gde tradicionalno nikada nisu imali željeni status. Od sredine 2017. godine u Evropskoj uniji je dozvoljeno sedam vrsta insekata za uzgoj i integraciju u hranu za akvakulturu, uz očekivano dalje širenje. Zato zapadni svet sve više pažnje posvećuje upotrebi, kao i kultivaciji ovih životinja. U radu su prikazani literaturni podaci u oblasti gajenja nekih insekata koji mogu da posluže za razvoj ove prakse i u evropskim uslovima.

Ključne reči: entomofagija, gajenje insekata, stočna hrana, hrana

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THE ASSOCIATION OF ROE DEER POPULATION WITH WEATHER CONDITIONS IN HUNTING AREA IN EASTERN CROATIA IN PERIOD 2008-2018

Mirna Gavran¹, Dragan Dokić², Maja Gregić¹, Vesna Gantner¹

¹ Faculty of Agrobiotechnical Sciences Osijek, University of Josip Juraj Strossmayer in Osijek, Vladimira Preloga 1, Osijek, Croatia

² Municipality Erdut, Bana Josipa Jelačića 4, Dalj, Croatia

Corresponding author: Mirna Gavran, mgavran@fazos.hr

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Abstract: Roe deer population response on climate change depends on availability of resources and the intensity of the change. The negative effect of cold winters on survival and reproduction and decreasing food availability affect population growth of roe deer. Air temperature is one of the main climatic elements when observing the climate of an area. Humidity is an important factor for normal life, it is calculated in percentages (up to 100%) and depends on the season. Climate conditions are important factors for the population dynamics of many ungulate species. They affect survival of offspring and strongly influence on reproduction. Considering the great importance of the roe deer population in Croatia, the aim of this study was to determine the knowledge about the relationship between all categories (offspring, young, middle-aged, and mature) of roe deer population and different weather conditions in hunting ground in Eastern Croatia during the analysed period from year 2008 to year 2018.

Key words: roe deer, population, humidity, air temperature

Introduction

Roe deer have expanded their range considerably in recent decades and are now found throughout Europe except for north Scandinavia, Iceland, Ireland and the Mediterranean islands (*Gaudry et al., 2018; Padie et al., 2015; Bonnot et al., 2017*). Further, they are also found in northern Asia across to China. Roe deer can tolerate a wide range of climates, from the dry heat of the Balkan karst to cold sites high in the Alps where they can be found at altitudes of up to 3000 meters, even in the depths of winter. They have also adapted well to living in cultivated areas, often close to human settlements, benefiting from intensive forestry and being relatively insensitive to air pollution. In accordance, to the literature (*Bagarić,*

2018; Budak and Pintur, 2017; Škvorc *et al.*, 2018) roe deer is widespread throughout Croatia with exception of some islands. Also, this population is more numerous in the mountainous and continental Croatia. According to Konjević (2008), the roe deer is divided into five groups by age: fawns, offspring, young, middle-aged and mature. Accordingly, to Tucak *et al.* (2006) the air temperature has a great influence on the vegetation of the natural hunting area, the water level of the water surface and the health of the wildlife in it. In most organisms the temperature of the body depends on the ambient temperature, which limits their spread to the biosphere. The temperature significantly affects the metabolism of wildlife. The influence of extreme temperatures on game health is apparent: large colds cause diseases of the digestive organs and organs of the urogenital system (kidneys, bladder and sex organs). The cause of these illnesses may be the use of frozen food in the hunting ground. On the other hand, the great heat may have the effect of drought that manifests itself in the destruction of vegetation, drainage of smaller water areas of bar and river tributaries. Such a condition forces the game to go because without food and water cannot survive. Humidity and water are important factors for normal life. Water is an integral part of all organisms and varies from one species to another. Snow represents an indirect danger to the wild game. The snow cover covers the soil and the flora and thus prevents the game from eating food and makes it difficult to move. This condition causes the metabolism disorder in the game, which can lead to death. During long-lasting snow-time periods, morphological anomalies are observed on the antlers of roe deer game, which are usually of passing character. In accordance to Sertić (2008), indirect influence of climate – at warmer climate the plants grow faster and better and the quality of the food leads to a higher quality trophy. Direct influence of climate – temperature, snow cover height, cloudiness, amount of sunlight, spring beginning and all meteorological events occurring during the development of antlers. The higher the snow, the roe deer harder comes to the food and automatically does not have enough mineral ingredients and bad antlers are created. This is not genetics or legacy; it is the influence of ecological factors. They affect the trophy value of the worthless throats and genetic good throats. The influence of temperature on the development of antlers - if the temperature for a longer period of time is extremely low and the antlers are in velvet, the antlers will freeze and for that year the trophy is worthless. Excessive amounts of precipitation can cause floods that are damaging many wildlife. Precipitation with strong winds can cause mortality on hairy and feathery young cubs in the early days of life. According to Tucak *et al.* (2001) in the formation of hunting grounds, migration of game related to climatic factors or conditions must always be planned. A game at certain times migrates - for example, during the summer heat it looks for cooler landscapes. The temperature is extremely important also for mating of roe deer.

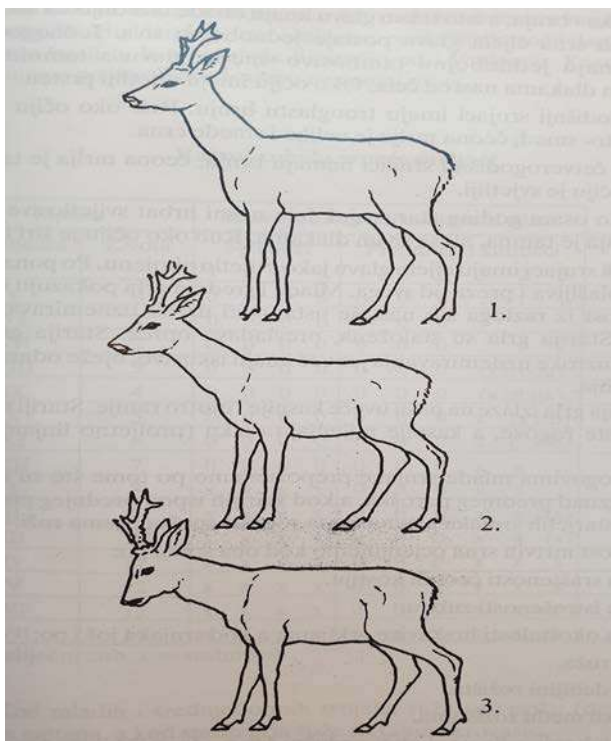
During mating they like great heat, the warmer it is, the mating is more intense. Considering the great importance of the roe deer population in Croatia, the aim of this study was to determine the knowledge about the relationship between all categories (offspring, young, middle-aged, and mature) of roe deer population and different weather conditions in hunting ground in Eastern Croatia during the analysed period from year 2008 to year 2018.

Materials and Methods

Accordingly, to *JAZU (1974)* the first written data on the hunting ground originated in 1721 when Karlo VI donated Baron Prandau land and woods south of the Drava River during the serving in the war against the Turks. In 1909, Count Pavle Pejačević killed a roebuck, whose trophy value was estimated at 158.30 CIC points. During the First World War and immediately afterwards, the poaching destroyed most of the wildlife funds. Also in 1938, wildlife funds, especially roe game, were affected by very long and sharp winter. By the Second World War, game funds have increased. According to the inventory of 01.01.1955 (*JAZU, 1974*) the following number of roe game was determined: 250 throats (90 bucks, 140 does and 20 fawns). During the Homeland War, game funds have also been devastated. (*JAZU, 1974*). The first dataset was collected in the hunting ground in eastern Croatia that makes a natural whole of 9364 hectares where the roe deer population has been intensively monitored for decades and the same populations of wildlife game resides in the whole area. The cultivated land surface in the hunting grounds is used for sowing the thickets for wild game, primarily grasslands, in order to raise the hunting area's nutritional capacity and the number of wild game. According to *Sertić (2008)*, in order to determine the climate of the hunting ground, it is necessary to know and analyse a few elements directly affecting it: precipitation, air temperature, wind, humidity and insolation. The organization of measurements and monitoring of the climatic phenomena is carried out in the network of meteorological stations. Meteorological stations are organizational units of meteorological services which have the task of performing meteorological observations (measurement and monitoring) according to the established unique criteria. A visual rating of a certain size is considered as observation, while the measurement refers either to the reading of the instrument value or to the registration of the appropriate type of instrument. The climate on the game is influenced indirectly and directly by the average and extreme temperatures, winds, insolation and the amount and shape of rainfall and humidity. Air temperature is one of the main climatic elements when observing the climate of an area. Given the temperature conditions, it is necessary to determine the annual strokes of this climatic element. Standard meteorological data on air temperature show an air temperature of 2 m above ground level. So, a range of 12 mid-monthly air

temperature values obtained from climatological measurements at 7, 14 and 21 hours is a long-term flow of temperature. The following values are followed: maximum daily, minimum daily, average monthly and average annual. The temperature is usually the lowest in January, followed by more or less regular increase until the hottest month (July). Each air temperature corresponds to the maximum amount of water vapour in which the air is saturated. Accordingly, the relative humidity of the air is a meteorological phenomenon that shows the percentage of air saturated with an aqueous vapour at a certain temperature of air. If, for example, the relative humidity of the air is 50%, in the air is only half the amount of water vapour that the air could contain at that temperature. Knowing this meteorological element is generally important because the formation of fog, cloud, rain, roses, frosts, and other hydrometeors depends on the amount of moisture in the air. In this paper, monthly precipitation sums, mean temperatures and average humidity were acquired for the respective region and periods. Data were provided by Croatian Meteorological and Hydrological Service.

The estimation of roe deer's age based on morphological characteristics is presented on Picture 1.



Picture 1. The estimation of roe deer's age, 1. young, 2. middle-aged, 3. mature (*Darabuš at al., 2002.*)

Results and Discussion

The relationship between weather conditions (air temperature and humidity) and population size of roe deer population in hunting ground in Eastern Croatia during the analysed period from year 2008 to year 2018 is shown in Figures 1 and 2.

During the analysed period from year 2008 till year 2018 the mean yearly air temperature varied in interval from 11 – 13°C. The highest number of offspring, of both gender, males and females, was recorded in year 2009, when mean air was 12°C. During the year 2017, when the mean air temperature amounted 11.5°C the smallest number of offspring, females and males was recorded. Furthermore, in year 2013, the highest number of young animal, males and females, was recorded, when the mean year ait temperature was higher comparing to other years and amounted 13°C. The smallest number of middle-aged animals, females and males, was determined in year 2017, while in the same year the smallest number of mature animals, males and females was determined. During the years 2008 and 2014 the mean yearly air temperature was slightly above 12°C.

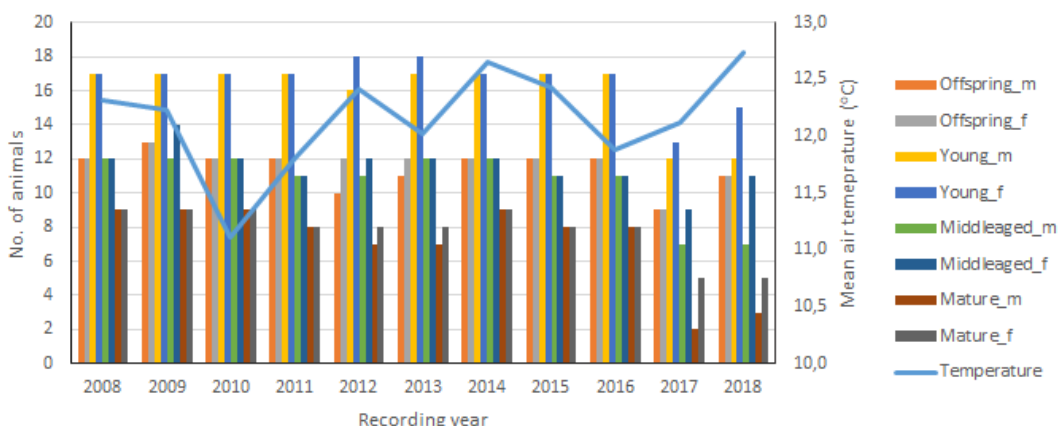


Figure 1. The influence of temperature on number of roe deer population in period 2008-2018.

In the researched hunting ground during the analysed period from year 2008 till year 2018 the mean yearly humidity varied in interval from 76 – 84 %. The

lowest mean humidity was recorded during the year 2012 when the number of offspring males was 10 and females 12. At the same year, number of female and male animals were determined, by category as follows: young males 16 and young females 18, middle-aged males 11 and females 12 and mature 15 animals. The highest mean yearly humidity was determined in years 2010 and 2014. In year 2017, the lowest number of mature animals, especially bucks were recorded.

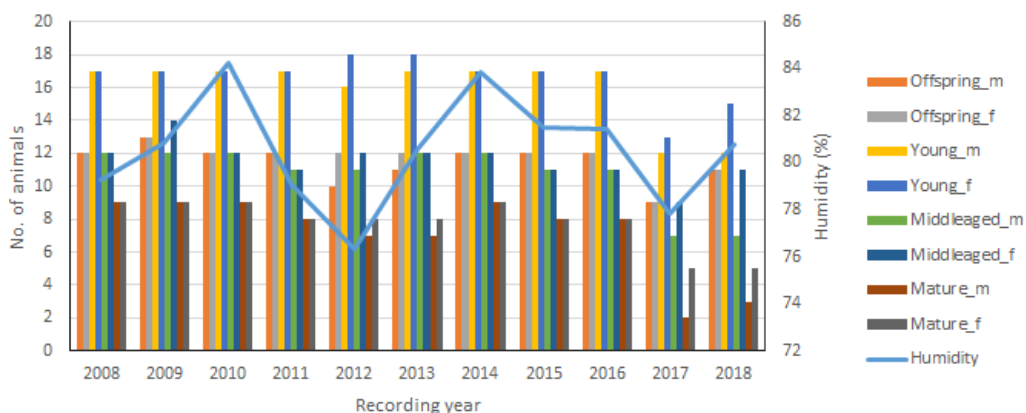


Figure 2. The influence of humidity on number of roe deer population in period from 2008-2018.

The same situation with the number of animals also occurred in air humidity, which means that the temperature and humidity of the air are closely related when it comes to the number of animals. For example, in 2017 at temperature of 10°C and 73% humidity, the smallest number of mature males was recorded. Climate conditions are known to be important factors for the population dynamics of many ungulate species. They affect juvenile survival and strongly influence reproduction. In accordance to *Plard et al. (2014)*, the lowered growth rate of roe deer seems to be attributable to recent climate change and is likely to get worse as the spring vegetation flush continues to advance with global warming. According to *Gaillard et al. (2013)*, the only way roe deer can escape the adverse effects of climate change is by increasingly shifting their distribution to open landscapes which offer richer food resources during spring, enabling females to successfully offset their energetic requirements during the critical rearing stage. This testable prediction is a promising avenue for future investigation.

Conclusion

Based on the conducted research following could be pointed out:

- during the analysed period from year 2008 till year 2018 the mean yearly air temperature varied in interval from 11 – 13°C,
- during the analysed period from year 2008 till year 2018 the mean yearly humidity varied in interval from 76 – 84 %,
- the biggest total number of offspring was determined in year 2009.

Since the offspring are extremely susceptible to unsuitable environmental conditions especially low temperature the decrease of roe deer population could be expected during the period characterised by extreme environmental conditions.

Povezanost populacije srna s vremenskim prilikama u lovištu u istočnoj Hrvatskoj u periodu 2008-2018

Mirna Gavran, Dragan Dokić, Maja Gregić, Vesna Gantner

Rezime

Reakcija populacije srna na klimatske promene zavisi od raspoloživosti resursa i intenziteta promena. Negativni uticaj hladnih zima na preživljavanje i razmnožavanje i smanjenje dostupnosti hrane utiču na rast populacije srna. Temperatura vazduha je jedan od glavnih klimatskih elemenata kada se posmatra klima neke oblasti. Vlažnost je važan faktor za normalan život, izračunava se u procentima (do 100%) i zavisi od godišnjeg doba. Klimatski uslovi su važni faktori dinamike populacije mnogih vrsta kopitara. Oni utiču na opstanak potomstva i snažno utiču na reprodukciju. S obzirom na veliki značaj populacije srna u Hrvatskoj, cilj ove studije bio je utvrditi znanje o odnosu između svih kategorija (potomstva, mladih, srednjih i zrelih) populacije srna i različitih vremenskih uslova u lovištima u istočnoj Hrvatskoj tokom analiziranog perioda od 2008. do 2018. godine.

Ključne reči: srna, populacija, vlaga, temperatura vazduha

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INFLUENCE OF MICROBIOLOGICAL PRODUCT BAIKAL EM1 ON THE DEVELOPMENT OF HYPOPHARYNGEAL GLANDS ON WORKER BEES AND THORACIC GLANDS ON WORKER BEES AND BEE DRONES

Rositsa Shumkova¹, Ralitsa Balkanska²

¹Research Center of Stockbreeding and Agriculture – Smolyan, 4700 Smolyan, Bulgaria

²Institute of Animal Science – Kostinbrod, 2232 Kostinbrod, Bulgaria

Corresponding author: Rositsa Shumkova, rositsa6z@abv.bg

Original scientific paper

Abstract: The aim of the study was to determine the influence of Baikal EM1 preparation on the development of hypopharyngeal glands (HPhGs) on worker bees and thoracic glands (ThGs) on worker bees and bee drones. The experiment was conducted during the period 23.04. – 10.06.2018 at Experimental apiary of Research Center of Stockbreeding and Agriculture – Smolyan, Bulgaria. The control group (5 bee colonies) was fed with sugar solution (sugar water 1:1) without additives and the experimental group (5 bee colonies) was fed with the product Baikal EM1 at a dose 10 ml/L sugar solution (sugar/water 1:1) 4 consecutive days at the beginning of the experiment. The bee colonies received Baikal EM1 have higher degree of development of the HPhGs than the control group. Worker bees and bee drones fed with Biakal EM1 had significantly higher height, diameter and volume of the ThGs than those from the control group bee colonies ($P < 0.001$). We expect our report to be a starting point for wider investigations, particularly aimed at a deeper understanding of development of ThGs.

Key words: hypopharyngeal glands (HPhGs), thoracic glands (ThGs), Baikal EM1, worker bees, bee drones

Introduction

Honeybees belong to insects with highly specialized feeding. Their food includes carbohydrates, protein, lipids, water, mineral elements and vitamins (Neupane and Thapa, 2005; Cho *et al.*, 2017). Biological indicators for evaluating the effect of stimulating feeding are amount of worker bee brood, development of

the glands, fat body, ovaries of the bee queen, life span of worker bees, some morphological characteristics such as live weight of the bee body and dimensions of the chitin parts. Nectar and bee honey are the main sources of carbohydrates in the natural diet of honey bees. Pollen is the primary source of protein, lipids, minerals and vitamins for bees (*Brodschneider and Crailsheim, 2010; Loidl and Crailsheim, 2001; Omar et al., 2017; Taha et al., 2019*). The proteins which bees received with the food are used for growing and development of the bee brood and young bees. In general, the honeybee salivary system comprises two secretory glands – the postcerebral gland and the thoracic gland (ThGs) (*Fujita et al., 2010*). Proteins are used for the development and secretory activity of hypopharyngeal glands (HPhGs) in worker bees. In this regard, the studies for influence of different protein additives and pollen substitutes are numerous (*Maurizio, 1954; Zhelyazkova, 1999; Zhelyazkova, 2000, 2001, 2013; Malone et al., 2004; Shumkova and Zhelyazkova, 2015; Shumkova, 2016*). The authors reported that the HPhGs are influenced by bee feeding and they could be taken as a criterion for assessing the beneficial value of the food supplements. Correlations between the degree of development of the HPhGs of worker bees and the amount of worker bee brood in the beehives could be found. The nurse's bees are characterized by highly developed HPhGs which are located in the bee's head and produce protein rich secretions such as royal jelly (*Hrassnigg and Crailsheim, 1998; Al-Ghamdi et al., 2011; Škerl and Gregorc, 2015*). The diameters of HPhGs could be used to describe the physiological status of worker honeybees. Furthermore, pollen consumption is positively correlated with gland development degree (*Hrassnigg and Crailsheim, 1998*).

ThGs are important for the bees feeding because their secretion is involved in digestion by activating the enzymes of the bee stomach. In addition, the gland secretion moisturizes the proboscis and dissolves the crystals of the carbohydrate food (crystal sugar, crystallized honey) (*Shumkova, 2016*).

Probiotics are widely used for the prophylaxis of infectious diseases and increase their resistance of pathogen organisms. In the beekeeping probiotics as additives demonstrate high productivity of the bee colonies (*Eremia et al., 2015*). The application of probiotics in beekeeping as an additive can increase the survival of bees and can improve their health status. Baikal EM1 is a multipurpose microbiological preparation created by Russian microbiologists. It consists of a complex of living beneficial microorganisms. The application of the preparation is universal for plants and animals (*Allahverdiyev et al., 2011; Lorets et al., 2015; Allahverdiyev et al., 2018*).

Literature data shows that studies on the effect of plant products on the development and secretory activity of the HPhGs and ThGs are relatively limited. In this respect the aim of the study was to determine the influence of Baikal EM1

preparation on the development of hypopharyngeal glands (HPhGs) on worker bees and thoracic glands (ThGs) on worker bees and bee drones.

Materials and Methods

The experiment was conducted in the spring period 23.04. – 10.06.2018 at Experimental apiary of Research Center of Stockbreeding and Agriculture – Smolyan, Bulgaria. Bee colonies of the local honeybee *Apis mellifera* L., settled in hives system Langstroth-Rut were used.

Spring feeding of the bee colonies

Two groups were formed – 1 control group (5 bee colonies) and 1 experimental (5 bee colonies), equal in quantity of bees (strength), brood and food supplies (honey and bee pollen). The control group was fed with sugar solution (sugar water 1:1) without additives. The experimental group was fed with the product Baikal EM1 at a dose 10 ml/L sugar solution (sugar/water 1:1) 4 consecutive days at beginning of the experiment. All bee samples were taken at the end of the spring feeding (12.06.2018).

Development of the HPhGs was determined in worker bees from each bee colony. The HPhGs were found with shallow cut in the head. They were removed and placed on glass slide on a drop of distilled water. Both lobes of the glands were evaluated. The degree of development of HPhGs was established using the 4-point scale of Hess by *Maurizio (1954)* with a binocular loupe LAB-20, OPTIKA-Italy.

Determination of the development of the ThGs of honeybees

ThGs of bees are located in front of the thorax and consist of elongated glandular cells in the form of tubes collected in cluster. The development of the ThGs was determined in 30 worker bees at the age of 5 – 10 days from each bee colony. For determining the degree of development of the ThGs of each bee, height and diameter of 10 glandular tubes (cylinders) are measured. As there are two variables (height and diameter), the volume of the tubes is calculated as volume of the cylinder according to the formula:

$$V = \pi \cdot r^2 \cdot h$$

where V is the volume of glandular tubes, π is 3.14, r is the radius of the glandular tubes and h is the height of glandular tubes.

For the measurement of the glands is used a binocular loupe LAB-20, OPTIKA-Italy.

The physicochemical parameters (water content, diastase, electrical conductivity) were determined according to the European Honey Commission recommended methods (*Bogdanov et al., 1997*).

Data was statistically evaluated with SPSS version 23 for Windows. The figures include mean values and standard deviations. Significance of differences were examined by Student's t-test. The significance was assumed for $P < 0.001$.

Results and Discussion

Maurizio (1954) classified the development of the HPhGs according to a scale from 1 to 4. The normal development of these glands (sizes of acini) is well known (*Crailsheim and Stolberg, 1989*). Furthermore, different feeding could have various effects on the HPhGs development in bees. The results for the degree of development of the HPhGs of worker bees are presented in Fig. 1. Bee colonies fed with Baikal EM1 have higher degree of development of the HPhGs than the control group. Until now the scientific information about the development of HPhGs and ThGs after microbiological product Baikal EM1 is insufficient. HPhGs were often used to describe the physiological status of worker honeybees.

The authors *Eremia et al. (2015)* used Bilaxan composed of microorganisms as a stimulating feeding during spring time. The strength of the bee colonies was increased by 5.56%, bee queens' prolificacy and sealed worker bee brood with 28.48 – 32.45% and productivity by 12.47%. The authors did not present results for the development of HPhGs.

Pătruică et al. (2013) examined the effects of prebiotic and probiotic products on intestines of working bees. The authors reported that intestinal development correlated with the absorption of nutrients. The bee colonies had good development during the active season.

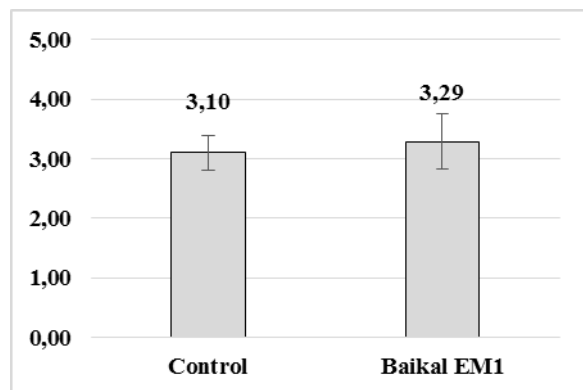


Figure 1. Degree of development of the HPhGs of worker bees (rates according to Hess)

Also, the results may suggest that Baikal EM1 can influence the secretion of the HPhGs. The treatment with probiotics could increase the number of larger acini while reducing the smaller acini of the HPhGs of worker bees. These morphological enhancements could increase royal jelly secretion by honeybees. Royal jelly is used to feed the larvae and bee queen.

For deeper understanding of HPhGs development honey samples from the two groups of bee colonies are collected. Table 1 shows the average values and standard deviations of water content, diastase activity and electrical conductivity of all analyzed honey samples. The water content is a useful parameter for describing moistness and viscosity of the honey. In Council Directive 2001/110/EC (2001) the maximum water content value of floral honey is given as not more than 20% in general. For all honey samples the water content is under this limit. It is well known that higher content can produce fermentation during storage (*Subramanian et al., 2007*).

Among the two observed groups statistically significant difference was in diastase activity ($P < 0.001$). Significant differences were not found in electrical conductivity (Table 1). In nursing bees HPhGs have a high secretory activity and they produce royal jelly. As the worker bees start to collect nectar and pollen their HPhGs decrease in size and produce enzymes involved in carbohydrate metabolism (*Klose et al., 2017*). The reason for the higher diastase in honey from the bee group which received Baikal EM1 could be due to well-developed HPhGs on the bee from the same group.

Table 1. Physicochemical parameters of bee honey (mean \pm SD)

Parameters	Control group (n=5)	Baikal EM1 (n=5)
Water content, %	17.50 \pm 0.50	17.80 \pm 0.76
Diastase activity, Gothe units	21.24 \pm 0.89***	23.58 \pm 0.75***
Electrical conductivity, mS/cm	0.845 \pm 0.030	0.834 \pm 0.045

SD – standard deviation

*** significant difference between Baikal EM1 and control group ($P < 0.001$)

In honey bees ThGs are salivary glands located in the thorax (*Martin et al., 2018*). The ThGs produce secretions mainly containing digestive enzymes that are involved in honey and sugar digestion. Also, ThGs are connected to pollen and wax moistening (*Feng et al., 2013*). They are developed in the bee queen, drones and honeybees. Until now the authors *Shumkova and Zhelyazkova (2015)* present results for the influence of stimulating products, plants extracts and electrochemically activated water solution the on the development of ThGs. The average values and standard deviations of the height and diameter of worker bees are shown in Fig. 2. In the present study the worker bees from the colonies given

the product Baikal EM1 had significantly higher height, diameter and volume than the control group ($P < 0.001$). (Fig. 2 and 3). When the ThGs are well developed they are more productive.

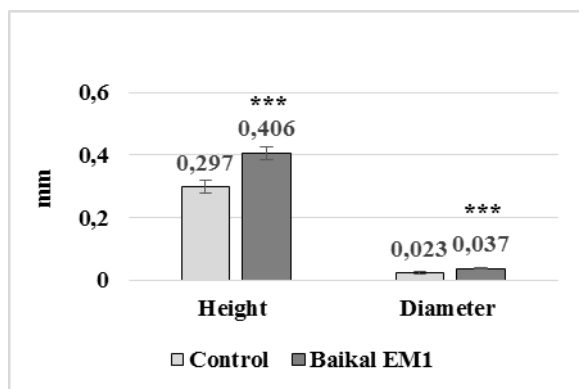


Figure 2. Mean values and standard deviations for height and diameter of the ThGs of worker bees (mm)

Legend: *** significant difference at $P < 0.001$

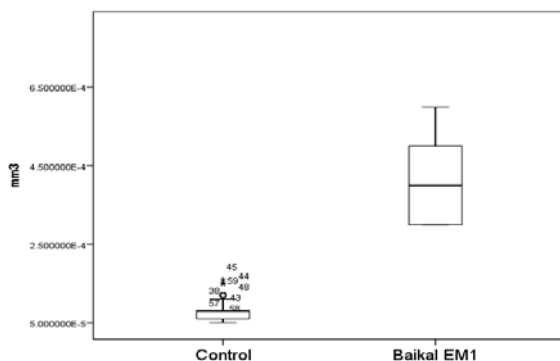


Figure 3. Box plot diagram of the volume of the glandular tubes (mm^3). For each group minimum, maximum and median values are shown for the ThGs of worker bees

Figure 3 shows glandular tubes of ThGs of worker bees (minimum and maximum values of the volume). To our knowledge, this is the first identification of ThGs of bee drones after feeding with microbiological product Baikal EM1. The height, diameter and volume of the ThGs of the bee drones were influenced by the Baikal EM1 supplementation and became significantly higher ($P < 0.001$) compared to the control group bee colonies (Fig. 4 and 5). The ranges of the height and diameter of the bee drones ThGs were as follows: control group 0.462 – 0.512 mm

and 0.030 to 0.040 mm; experimental group 0.594 – 0.634 mm and 0.034 – 0.043 mm, respectively.

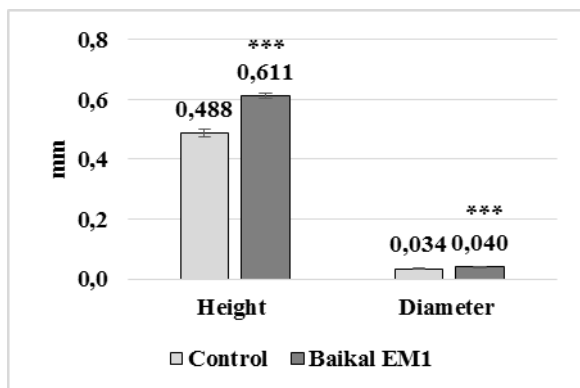


Figure 4. Mean values and standard deviations for height and diameter of the ThGs of bee drones (mm)

Legend: *** significant difference at $P < 0.001$

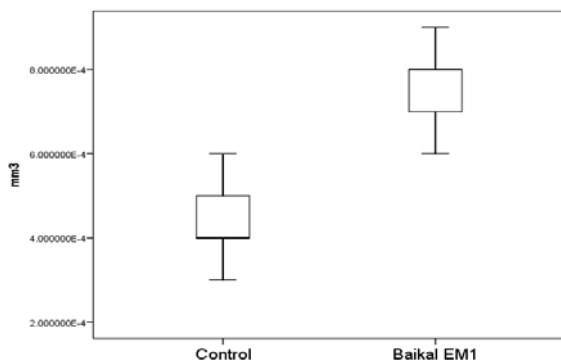


Figure 5. Box plot diagram of the volume of the glandular tubes (mm³). For each group minimum, maximum and median values are shown for the ThGs of bee drones

Conclusion

Bee colonies fed with Baikal EM1 have higher degree of development of the HPhGs than the control group. Worker bees and bee drones fed with Biakal EM1 had significantly higher height, diameter and volume of the HPhGs than those from the control group bee colonies ($P < 0.001$). We expect our report to be a starting point for wider investigations, particularly aimed at a deeper understanding of development of ThGs after different bee feeding.

Uticaj mikrobiološkog proizvoda Baikal EM1 na razvoj hipofaringealnih žlezda pčela i torakalnih žlezda pčela i trutova

Rositsa Shumkova, Ralitsa Balkanska

Rezime

Cilj studije bio je utvrditi uticaj preparata Baikal EM1 na razvoj hipofaringealnih žlezda (HPhG) pčela radilica i torakalnih žlezda (ThG) pčele radilice i trutova. Eksperiment je sproveden u periodu 23.04. - 10.06.2018. godine, u eksperimentalnom pčelinjaku Istraživačkog centra za stočarstvo i poljoprivredu - Smoljan, Bugarska. Kontrolna grupa (5 pčelinjih kolonija) hranjena je rastvorom šećera (šećerna voda 1: 1) bez dodataka, a eksperimentalna grupa (5 pčelinjih kolonija) hranjena je proizvodom Baikal EM1 u dozi od 10 ml/L rastvora šećera (šećer / voda 1: 1) 4 uzastopna dana na početku eksperimenta. Pčelinje porodice koje su primile Baikal EM1 imale su veći stepen razvoja HPhG od kontrolne grupe. Pčele radilice i trutovi hranjeni Baikalom EM1 imali su značajno veću visinu, prečnik i zapreminu ThG od onih iz kontrolnih grupa pčelinjih kolonija ($P < 0,001$). Očekujemo da će naš izveštaj biti polazna tačka za šira istraživanja, posebno usmerena na dublje razumevanje razvoja ThG.

Ključne reči: hipofaringealne žlezde (HPhG), torakalne žlezde (ThG), Baikal EM1, pčele radilice, pčelinji trutovi

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SIGNIFICANCE OF INVESTMENTS IN AGRICULTURAL PRODUCTION ON THE EXAMPLE OF THE RURAL COUNTIES OF THE REPUBLIC OF CROATIA

Dragan Dokić¹, Maja Gregić², Mirna Gavran², Vesna Gantner²

¹Municipality Erdut, Bana Josipa Jelačića 4, Dalj, Croatia

²Faculty of Agrobiotechnical Sciences Osijek, University of Josip Juraj Strossmayer in Osijek, Vladimira Preloga 1, Osijek, Croatia

Corresponding author: Dragan Dokić, dragan.dokic79@gmail.com

Original scientific paper

Abstract: Local economic processes represent social processes that should contribute to local development. These processes are complex and need to be meticulously accessed. They include the stages of preparation, planning, organization, implementation, management, monitoring and control. The aim of this paper was to show how in practice the investment affects the volume of agricultural production. Research was conducted on the plant production in Osijek-Baranja, Vukovar-Srijem and Brod-Posavina County. Through the capital coefficient, the level of engagement of resources in agricultural production will be shown. Furthermore, economic development must be accompanied by the investment process. From a local aspect, investments have an impact on the increase in production of goods and services, what will be shown in the paper. Increasing the amount of investment on one hand is determined by increasing capital equipment, but on the other hand it must be accompanied by investment spending. That is why it is necessary to show the economic efficiency of investments in agricultural production, which will be done through the marginal capital coefficient and marginal efficiency coefficient. These two coefficients will show an increase in agricultural production with the aim of creating the preconditions for a sustainable state of agricultural production.

Key words: Investments, plant production, capital coefficient, marginal capital coefficient, marginal coefficient of effectiveness

Introduction

Local development and achievement of defined goals is unimaginable without the investments. The concept of economic development includes an increase in production not only at the local level of economy, but also wider, which

explains complex transformations in the composition and structure of the economy, as well as changes in significant inputs and their shares in growth of total output (*Porter 2000*). The eastern region of the Republic of Croatia abounds in viable agricultural land, which is a relative advantage over other regions. The concept of relative advantages in practice implies that one area concentrates on the production of those types of goods for which there is less opportunity cost. In Osijek-Baranja, Vukovar-Srijem and Brdo-Posavina County, plant production is a strategic developmental branch. However, the competitiveness of production is difficult to maintain without investment. Local agricultural producers who have no investment, they literally maintain only the current reproduction, which can have negative consequences in the long run. Therefore, investments should focus on stimulation of production, employment and aggregate demand, i.e. to put them into the function of capital accumulation (*Litterman, 2003*). The increase in capital investment in buildings, equipment, infrastructure and so on, raises business potential (*Dunning, 1972*). The term investment may indicate any investment, primarily funds, materials, equipment, and so on, all in order to obtain certain economic benefits or profits (*Ćirović, 2008*). Thereby, from the aspect of local politics, the most significant investments are the one investing in real forms of property that enable the realization of economic benefits through certain productive business activities. Investments are the determinants of economic development, which gains great significance at the local level. The investment, in everyday practice, implies (*Jovanović, 2003*):

- Funds invested in a particular business activity,
- Process of transformation of funds into production goods, i.e. the process of investing in productive goods and
- The subject of investing and obtaining as a result of the investment process.

The investment process indicates changes (*Hirt, 2005*). In order for the changes to be treated as developmental, it is necessary that the result of these changes be a positive economic effect, i.e. it is necessary for these changes to achieve a positive growth (*Greym, 2008*). If such changes do not produce a positive growth, then they do not have the character of developmental, but lead to stagnation, and later to the dystrophy and system failure. Unfortunately, such a scenario is evident in the rural areas of the Republic of Croatia, which is a reflection of inadequate access to the process of considering the importance of investment. Investment as a process enables the growth of a potential product and creates conditions for achieving economic development (*Pike, 2009*). In accordance to *Sisek (2005)* there are five basic conditions for attracting foreign direct investments as follows:

- Political stability,
- Stability of the national economy,
- Affordable business environment,
- Infrastructure building,
- Credibility of government policy.

Since investments are a complex area, various indicators and calculation methods are used for analysis. The effectiveness of the investment is expressed by the relationship between capital (basic material funds) and production or the relationship between investment and production. In other words, the economic effectiveness of the investment essentially reflects the relationship between the consumption of capital spending and their production effect. The quantitative expression of the *capital coefficient*, k , is expressed by the following equation (Vukadinović, 2015):

$$k = \frac{K}{Y}$$

where:

- K – production funds;
- Y – production output;
- k – capital coefficient.

The capital coefficient expresses the degree of supply of production by capital, i.e. shows how many units of capital come to the unit of production, and accordingly, what is the production effect of each unit of capital. Furthermore, the economic effectiveness of the investment is expressed by the marginal capital coefficient and marginal coefficient of effectiveness.

Marginal capital coefficient, k' , or *limitary capital coefficient* represents the relationship between investment and production growth. This coefficient shows how many units of investment need to be invested in order to generate a growth of unit of production expressed as the unit of social gross product. The marginal capital coefficient is expressed by the following equation (Joens, 2007):

$$k' = \frac{K_1 - K_0}{Y_1 - Y_0}$$

where:

- k' – marginal capital coefficient,

- K_1 – production in the current year,
- K_0 – production in the previous year,
- Y_1 – investments in the current year,
- Y_0 – investments in the previous year.

The marginal coefficient of effectiveness, e' , is the reciprocal form of marginal capital coefficient expressed by the following equation (Joens, 2007):

$$e' = \frac{Y_1 - Y_0}{K_1 - K_0}$$

The investments cannot be realized without the appropriate investment policy that defines the appropriate goals beforehand, after which investment projects are selected and finally their realization. Smart investment policy is necessary because of the limited resources with which every economy faces (Jović and Vukadinović, 2012).

Material and Methods

In order to apply theoretical models and analyse the investments in plant production, data from the State Bureau of Statistics of the Republic of Croatia regarding the volume of production funds, production value and investments in agriculture – plant production (Table 1) were analysed. Data were expressed in thousands of euros. Analysed data refer to three counties: Osijek-Baranja, Vukovar-Srijem and Brod-Posavina. Those counties belong to the rural area, have similar production potentials and are approximate in size.

Table 1. The production funds, production results and investments in production (in 000 Eur) in analysed counties

Year/County	Vukovar-Srijem county	Osijek-Baranja county	Brod-Posavina county
	Production funds		
2013.	4.866.144,00	7.348.684,57	3.521.209,71
2014.	5.588.802,51	9.187.322,40	4.820.010,00
2015.	6.356.531,66	10.077.560,00	5.402.996,40
2016.	8.678.756,57	11.019.060,00	7.240.428,00
2017.	8.808.069,60	11.896.665,49	7.214.695,71
Year/County	Production result		
2013.	1.390.326,86	1.837.171,14	1.173.736,57
2014.	1.433.026,29	1.997.244,00	1.377.145,71
2015.	1.444.666,29	2.015.512,00	1.317.804,00
2016.	1.446.459,43	1.695.240,00	1.392.390,00
2017.	1.398.106,29	1.830.256,23	1.311.762,86
Year/County	Investments in production		
2013.	196.228,20	177.820,08	163.619,56
2014.	306.413,86	280.233,33	186.718,84
2015.	312.287,69	326.494,35	174.931,71
2016.	354.030,95	292.832,26	178.433,32
2017.	396.654,02	307.668,40	215.628,14

Based on data presented in Table 1 following coefficients, as economic indicators of the effectiveness of the business, were calculated:

- the capital coefficient,
- the marginal capital coefficient and
- the marginal coefficient of effectiveness.

Results and Discussion

The capital coefficient, the marginal capital coefficient and the marginal coefficient of effectiveness of plant production in analysed counties (Vukovar-Srijem, Osijek-Baranja, and Brod-Posavina) in the period from year 2013 until 2017 is presented in the Table 2.

Table 2 The capital coefficient, the marginal capital coefficient and the marginal coefficient of effectiveness in analysed counties in the period from year 2013 until 2017

Year/County	Vukovar-Srijem county	Osijek-Baranja county	Brod-Posavina county
	Capital coefficient		
2013.	3.5	4.00	3.00
2014.	3.9	4.60	3.50
2015.	4.4	5.00	4.10
2016.	6.0	6.50	5.20
2017.	6.3	6.50	5.50
	Marginal capital coefficient		
2013.	0.00	0.00	0.00
2014.	0.39	3.85	4.39
2015.	1.98	0.49	1.70
2016.	0.15	-3.74	3.17
2017.	-0.67	-2.40	-4.69
	Marginal coefficient of effectiveness		
2013.	0.00	0.00	0.00
2014.	2.58	0.26	0.23
2015.	0.50	2.03	0.59
2016.	6.55	-0.27	0.32
2017.	-1.50	-0.42	-0.21

The calculated marginal capital coefficients in Vukovar-Srijem county was negative in year 2017, in Brod-Posavina county also in year 2017, while in Osijek-Baranja county negative value of marginal capital coefficients was recorded in 2016 and 2017. Same signs of marginal coefficient of effectiveness was recorded. The negative sign of both coefficients means that as investment decreases, production decreases, and vice versa, a positive sign, means that investments and growth of production have a positive mutual effect. The average marginal capital coefficient and average marginal effectiveness coefficient in analysed counties is presented in the Figure 1. The negative sign of average marginal capital coefficient recorded in Vukovar-Srijem County means that as investment decreases, production decreases and investments are reduced. In both Osijek-Baranja County and Brod-Posavina County, both calculated coefficients have positive sign, meaning that investments and growth of production have a positive mutual effect.

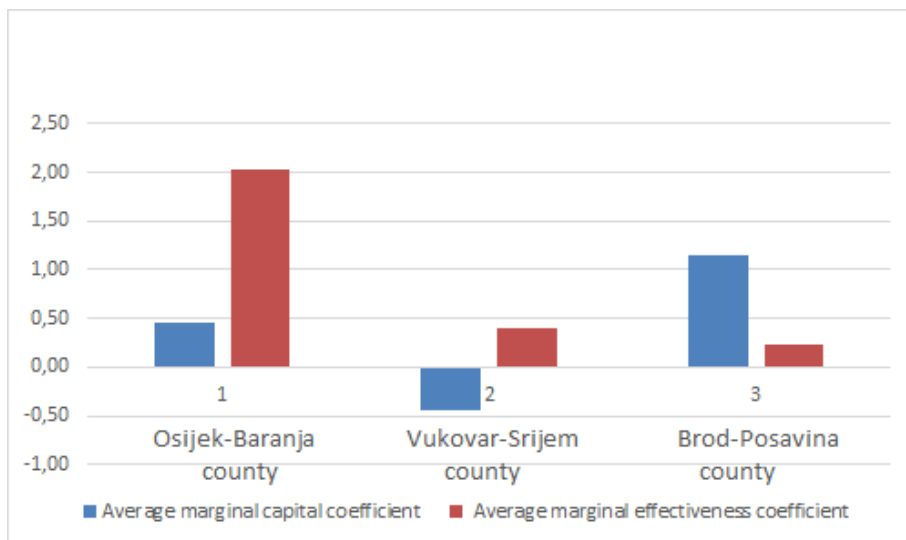


Figure 1. Comparison of average marginal capital coefficient and average marginal effectiveness coefficient in analysed counties

By placing the ratio of the average marginal capital coefficient and the average marginal coefficient of effectiveness for the analysed counties, a correlation value of -0.0023 is obtained which implies a negative correlation that indicates a negative correlation between these two coefficients. The growth of the marginal capital coefficient does not have to be accompanied by increases in the marginal coefficient of effectiveness.

Conclusion

Analysis of the situation of plant production as a strategic branch in Osijek-Baranja, Vukovar-Srijem and Brod-Posavina County, through investment approach should be viewed with caution. This is a production whose conditions apart from markets, instruments of fiscal policy and local economic policy are also determined by meteorological conditions. The paper analysed the five-year period 2013 - 2017, in which meteorological conditions were not optimal and favoured by agricultural producers. Therefore, there were evident oscillations in the volume of production. Regardless of this, investment factor should not be neglected, especially at the local level. Local governments need to focus their economic policy on creating a stimulating investment environment to improve the quality of life in their area and to stimulate the development of agricultural production.

Značaj ulaganja u poljoprivrednu proizvodnju na primeru ruralnih županija Republike Hrvatske

Dragan Dokić, Maja Gregić, Mirna Gavran, Vesna Gantner

Rezime

Lokalni ekonomski procesi predstavljaju društvene procese koji treba da doprinesu lokalnom razvoju. Ovi procesi su složeni i njima se mora pažljivo pristupiti. Oni uključuju faze pripreme, planiranja, organizacije, implementacije, upravljanja, nadgledanja i kontrole. Cilj ovog rada bio je da se pokaže kako u praksi investicija utiče na obim poljoprivredne proizvodnje. Istraživanje je sprovedeno na biljnoj proizvodnji u Osječko-Baranjskoj, Vukovarsko-Sremskoj i Brodsko-Posavskoj županiji. Kroz koeficijent kapitala biće prikazan nivo angažovanosti resursa u poljoprivrednoj proizvodnji. Nadalje, ekonomski razvoj mora biti praćen investicionim procesom. Sa lokalnog aspekta, ulaganja imaju uticaja na povećanje proizvodnje dobara i usluga, o čemu će biti reči u radu. Povećanje iznosa ulaganja s jedne strane određuje se povećanjem kapitalne opreme, ali s druge strane mora biti praćeno investicionom potrošnjom. Zato je neophodno pokazati ekonomsku efikasnost ulaganja u poljoprivrednu proizvodnju, što će se postići preko graničnog koeficijenta kapitala i graničnog koeficijenta efikasnosti. Ova dva koeficijenta će pokazati povećanje poljoprivredne proizvodnje sa ciljem stvaranja preduslova za održivo stanje poljoprivredne proizvodnje.

Ključne reči: investicije, biljna proizvodnja, koeficijent kapitala, granični koeficijent kapitala, granični koeficijent efikasnosti

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POSTER SECTION II

INSULIN RESISTANCE IN DAIRY COWS

**Radojica Djoković¹, Zoran Ilić², Marko Cincović³, Vladimir Kurćubić¹,
Miloš Petrović¹, Milan P. Petrović⁴, Violeta Caro Petrović⁴**

¹University of Kragujevac, Faculty of Agronomy, Department of Animal Science, Cara Dušana 34, Čačak, Serbia

²University of Priština, Faculty of Agriculture, Department of Animal Science, Kopaonička bb, Lešak, Serbia

³University of Novi Sad, Faculty of Agriculture, Department of Veterinary Medicine, Trg D. Obradovića 8, Novi Sad, Serbia

⁴Institute for Animal Husbandry, Autoput 16, Belgrade-Zemun, Serbia

Corresponding author: Radojica Đoković, radojicadjokovic@gmail.com

Review paper

Abstract: Insulin resistance is a state in which the biological effect of insulin is reduced when its concentration decreases or when a compensatory mechanism increases its concentration. Insulin resistance is characterized by reduced insulin response to glucose, i.e. decreased pancreatic beta cell function (insulin hyporesponsiveness) and/or reduced sensitivity of glucose to insulin (reduced intake of glucose by peripheral tissues under the influence of insulin – *eng.* insulin sensitivity). Methods for estimating insulin resistance include direct methods (hyperinsulinaemic-euglycaemic clamp, golden standard), indirect methods (intravenous glucose tolerance test) and surrogate methods (indices calculated from basal concentrations of insulin, glucose, non-esterified fatty acids (NEFA) and β -hydroxybutyrate (BHB), (HOMA, QUICKI, RQUICKI and RQUICKI- BHB). Surrogate indices show correlations with direct and indirect test results but they are inconsistent. Inconsistency occurs because the dependence of glucose concentrations on the degree of hepatic gluconeogenesis should be kept in mind when evaluating insulin resistance in ruminants. Therefore, the hyperinsulinaemic-euglycaemic clamp method is particularly suitable as it excludes gluconeogenesis in hepatocytes from analysis. Our results have shown a correlation between HOMA, QUICKI and RQUICKI indices and metabolic profile parameters. The correlation between dynamic and basal responses of NEFA, BHB, insulin, glucose and inorganic phosphorus is significantly dependent on RQUICKI-BHB index values in ketotic cows. RQUICKI index of insulin resistance is

substantially dependent on NEFA values in early lactation as well as on insulin and glucose values in the dry period.

Keywords: dairy cows, insulin resistance, HOMA, QUICKI, RQUICKI, RQUICKI- BHB

Introduction

Parturition and lactogenesis are accompanied by many physiological changes that facilitate the maintenance of homeostasis. This involves changes in the activity of almost all cells in the organism in order to satisfy the needs of the mammary gland through nutrient redistribution. Adaptation of the endocrine system (homeorhesis) during the transitional period is the key factor in maintaining metabolic balance (*Bauman and Currie, 1980; Aceves et al., 1985*). Growth hormone (GH) concentration increases at this time; this increase is accompanied by an increase in IGF and IGF binding proteins in mammary secretions, suggesting a role for these factors in mammary development and lactogenesis. However, as one of the homeorhetic complex of hormones, GH would be sensitive to changes in nutrient supply and these might modify its effects on mammary development (*Bauman and Currie, 1980; Batth et al., 2012*). Similarly, plasma concentrations of insulin, another homeorhetic hormone, would be changed by prepartum nutrition and this would affect nutrient supply to the udder (*Bauman and Currie, 1980; Balogh et al., 2008; Djokovic et al. 2014, 2015a, 2015a*).

Insulin plays a role in the adaptation of organic matter metabolism in dairy cows during the transitional period and during lactation, particularly in terms of nutrient redistribution and partitioning towards the mammary gland (insulin resistance) (*Balogh et al., 2008*). Insulin concentrations tend to decrease in early lactation, particularly in higher yielding cows. Thereafter, plasma insulin concentrations are relatively low and remain so throughout lactation, thereby facilitating the mobilization of organic matter from body reserves and its effective use in the synthesis of milk components (*Balogh et al., 2008; Djokovic et al., 2014, 2015a, 2015b*).

Insulin resistance is a phenomenon that occurs in early lactation and is characterised by lower insulin production and lower tissue response to insulin. It allows two important processes: conservation of glucose for lactose production in milk and increased lipolysis followed by increased NEFA concentration as energy purpose for high milk production (*Holtenius and Holtenius, 2007; Djokovic et al., 2014, 2015a*).

Insulin resistance involves a decrease in insulin responsiveness, i.e. pancreatic beta-cell function, and/or insulin sensitivity, i.e. glucose entry into tissues through the action of insulin (Kahn, 2006; Holtenius and Holtenius, 2007). Insulin resistance during the periparturient period is needed to the udder, as an organ in which glucose utilization is not insulin-dependent, given enough nutrients and energy to initiate lactation. These changes are related to milk production initiation, reduced feed intake, negative energy balance, as well as endocrine and metabolic changes at calving (Hayirli, 2006; De Koster and Opsomer, 2013).

Insulin resistance can be determined by numerous methods, such as direct, indirect and surrogate methods (Singh and Saxena, 2010). Surrogate methods are indexes calculated from the basal concentration of insulin, glucose and/or NEFA, and include the following indexes: HOMA (*Homeostatic Model Assessment*), QUICKI (*Quantitative Insulin Sensitivity Check Index*) and RQUICKI (*Revised Quantitative Insulin Sensitivity Check Index*). Indexes of insulin resistance relevance have been estimated by comparison with results obtained by hyperinsulinemic euglycemic clamp as the golden method for determination of insulin resistance (Holtenius and Holtenius, 2007; Balogh et al., 2008; Cincovic et al., 2014b, 2017, Djokovic et al., 2017, Alves-Nores et al., 2017).

It is known that some metabolic adaptations that characterize the early lactation period, are in relation with insulin resistance. These adaptations are increased lipid mobilization, ketogenesis and lipid oxidation, reduced levels of thyroid hormones, changed GH to IGF-I ratio, increased levels of cortisol, changes in hepatic insulin receptors abundance, increased activity of hepatic enzymes and reduced concentration of Ca and P (Djokovic et al., 2014, 2015a). However, there is a lack of literature data related to the direct relationship between the value of the indexes of insulin resistance and metabolic or endocrine parameters.

The aim of this study was to examine phenomenon of insulin resistance in dairy cows and determine the relations between indices of measurement of insulin resistance (HOMA, QUICKI, RQUICKI and RQUICKI-BHB).

Role of insulin secretion in dairy cows

Insulin is the main hormone in all adaptive processes during metabolic stress in peripartal cows. Research on insulin resistance has become a crucial point in understanding peripartal metabolism. Insulin has a major influence on nutrient partitioning to particular tissues and mammary glands. Insulin is an antilipolytic and antiketogenic hormone that has a major role in appetite regulation. Insulin is a

polypeptide constructed from amino acids connected by disulfide bonds and it is secreted from Langerhans cells of the pancreas. (*Pierzynowski and Zabielski, 1999; Balogh et al., 2008*).

Insulin actions at the cellular level are mediated by mRNA transcription and translocation, which affect the metabolism of glucose, proteins and fats (*Wilcox, 2005; Hayirli, 2006*). Glucose enters in the cell through different glucose transport proteins (GLUT) which can act without the presence of insulin (GLUT1 in the brain and erythrocytes; GLUT2 in tubular cells, epithelial cells of the small intestine and the liver, GLUT3 in neurons and placenta) or with insulin action (GLUT4 fat tissue, muscle tissue). As regards carbohydrate metabolism, insulin increases the production of glycogen and reduces glycogenolysis by dephosphorylation of glycogen synthase i.e. glycogen- phosphorylase. Insulin increases the conversion of pyruvate to acetyl CoA by activation of the intramitochondrial pyruvate dehydrogenase complex. Acetyl CoA can further be oxidized in the Krebs cycle or fatty acids can be synthesized. Another aspect of insulin action is that insulin stimulates the synthesis of fatty acids in adipose tissue. Fatty acid synthesis increases with increasing phosphorylation of acetyl-CoA carboxylase. Suppression of fatty acid oxidation is allowed by carnitine-acyltransferase inhibition. Insulin induces protein synthesis in many tissues. It is in charge of mRNA transcription and mRNA translocation in ribosomes. Examples of increased mRNA transcription include mRNA for glucokinase, phosphokinase, synthesis of fatty acids and albumins in the liver and pyruvate carboxylase in adipose tissue. Insulin reduces mRNA for hepatic enzymes, such as carbamoyl phosphate synthetase, which is the main enzyme in the urea cycle. Insulin is in charge of the synthesis of many growth factors, such as IGF-I (*Pierzynowski and Zabielski, 1999; Wilcox, 2005; Hayirli, 2006*).

There are many factors that affect insulin secretion in cows. Stimulators are: glucoses, galactose, xylitol, glyceraldehyde, many amino acids, fatty acids, sodium, vagus stimulation, calcium, gastrointestinal hormones (glucagon, cholecystokinin, pancreatic peptides, secretin) and some medications. Factors that cause insulin suppression are: greater body mass (related to increased lipolysis and higher concentrations of lower fatty acids in the blood), gastrointestinal hormones (galanin and somatostatin), sympathetic activity (hormones produced by the adrenal glands), inflammation mediators and full hormones (*Pierzynowski and Zabielski, 1999; Wilcox, 2005; Hayirli, 2006*). Many of these factors together affect insulin secretion in the periparturient period. Beta pancreatic cells have IGF-I and insulin receptors, which can allow additional insulin secretion after an increase in blood glucose levels. Insulin allows beta cells to detect glucose levels and regulates growth and regeneration of beta cells (*Kulkarni, 2004*).

Considering reduced feed intake and negative energy balance as main adaptation processes in early lactation related to insulin resistance, the degree to which insulin reduces body condition score and feed intake should be analyzed (Anil *et al.*, 1980; Deetz and Wangsness, 1980; Foster *et al.*, 1991).

In peripheral insulin administration, glucose concentrations were reduced (because glucose enters cells), which is a main factor that contributes to reduced feed intake. Given that the administration of glucose and insulin prevents hypophagia (Haupt, 1994), infusions of insulin antibodies increase appetite (McGowan *et al.*, 1992). Prolonged administration of glucose and insulin during the hyperinsulinaemic-euglycaemic method for evaluating insulin resistance decreases feed intake (McGuire *et al.*, 1995; Griinari *et al.*, 1997; Annen *et al.*, 1998; Marcle *et al.*, 1999). These effects of insulin can be of significance for cows in the dry period, but its influence on appetite in early lactation is probably not significant because its concentration is reduced. Interestingly, feed intake in cows with greater body condition scores is significantly reduced after calving. In these cows, insulin concentration is greater in the peripartal period (Holtenius *et al.*, 2003; Roche *et al.*, 2009; Bobowiec *et al.*, 2011).

Phenomenon of insulin resistance in dairy cows

Insulin resistance is a state in which the biological effect of insulin is reduced when compensatory mechanisms induce an increase in its concentration (Cefalu, 2001). Insulin resistance is characterized by reduced insulin responsiveness to glucose i.e. reduced function of beta pancreatic cells (insulin hyporesponsiveness) and/or reduced sensitivity of glucose to insulin i.e. reduced entrance of glucose to tissues under the influence of insulin (insulin sensitivity) (Kahn, 2006). Insulin resistance can be caused by prereceptor abnormalities (reduced secretion and/or increased insulin degradation), receptor abnormalities (reduced number of receptors and/or reduced affinity of receptors for insulin) and post-receptor abnormalities (defects in cellular signalization and translocation of glucose transporters) (Hayirli, 2006).

Insulin resistance in the peripartal period is necessary for the udder, an insulin-independent organ, to have enough nutrients and energy for lactation which starts. The phenomenon of insulin resistance is most pronounced after calving. Response of insulin to glucose is reduced and clearance of glucose is greater in post-partal period than in pre-partal period (Holtenius *et al.*, 2003; Bossaert *et al.*, 2008; Djokovic *et al.*, 2009).

During lactation, the udder expresses insulin independent glucose transporters, even three times greater than in the dry period. These receptors were found in fat

tissue in cows during late lactation and dry period but not in cows in peak lactation. Activation of insulin sensitive receptors in skeleton muscles and fat tissue remains unchanged during lactation or in the dry period (*Komatsu et al.*, 2005). In addition to reduced insulin response to glucose, insulin sensitivity of peripheral tissues is unchanged but greater insulin clearance rates in mid lactation have been found compared to the dry period (*Sano et al.*, 1993). Reduction in insulin sensitivity of tissues was found in a study where hyperinsulinaemic-euglycaemic clamp was used (*Mashek et al.*, 2001).

Malnutrition and reduced feed intake reduce insulin secretion and insulin response to glucose (*Meadler et al.*, 2001). Cows fed reduced nutrients showed a low insulin response after glucose administration even two weeks after calving compared to cows fed *ad libitum*. Nutrients, especially fats, can affect the degree of insulin resistance. Therefore, short-term hyperlipidaemia caused by the intravenous administration of sterile fat increases the basal concentration of glucose and insulin with reduced clearance of glucose after a load test. However, some fatty acids (from linseed oil) can be used for improvement of insulin sensitivity and antilipolytic effect of insulin in fat tissue (*Pires et al.*, 2007a, 2007b, 2008).

Zhang et al. (2013) noted that the intake of high-energy feed before calving reduces the expression of genes for insulin receptors in fat tissue during the postpartal period. Genetic predisposition and productivity of cows are significant factors that influence the development of insulin resistance. High-selection cows and cows that produce high milk amounts showed significantly greater insulin resistance and a slow reduction of glucose concentrations at the basal level after the glucose tolerance test (*Swali and Wathes*, 2006; *Chagas et al.*, 2009).

Effects of insulin resistance are dependent on the number of tissues showing insulin resistance at a given moment. This mainly refers to fat, muscle and liver tissue. In fat tissue, insulin resistance causes a reduction in lipogenesis and an increase in lipolysis. In the peripartal period, blood NEFA concentrations are increased due to lipid mobilization. Higher NEFA concentrations are related to reduced insulin sensitivity of fat tissue in cows that are not in lactation or to reduced clearance of glucose and insulin after glucose load in dairy cows (*Bossaert et al.*, 2009; *Oikawa and Oetzel*, 2006).

High NEFA concentrations depress insulin-stimulated glucose uptake in skeletal muscles and suppress gluconeogenesis in the liver (*Hayirly*, 2006). Laboratory mouse models showed a direct influence of NEFA on beta pancreatic cells (*Meadler et al.*, 2001). Speaking of muscle tissue, it has been known that insulin induces a suppression of protein catabolism while insulin deficiency can cause protein catabolism in order to use amino acids for gluconeogenesis. In insulin resistance, synthesis of glycogen in muscles is reduced due to reduced intracellular translocation of glucose. An insulin dose high enough to increase

protein anabolism is much higher than the dose of insulin that protects the catabolism of existing proteins. Based on this, it is obvious that insulin has a protective role.

Glucose in the liver is used by insulin-independent receptors but the presence of insulin is necessary to allow crucial metabolic processes. Insulin induces glycogen synthesis, while reducing gluconeogenesis and synthesis of ketone bodies. Mitotic effects of insulin (and growth hormone) are reflected in IGF-I production. In a state of insulin deficiency, such as starvation, all processes can be equally affected.

However, under conditions of insulin resistance, these changes are not always equal. For example: insulin resistance in starvation can cause increased gluconeogenesis, but under classic insulin resistance, when compensatory insulin concentrations increase, the mitotic action of insulin on hepatocytes is present and IGF-I concentration increases. Disturbance in lipoprotein metabolism is an important indicator of insulin resistance in hepatocytes. Greater concentrations of NEFA entering the liver with reduced catabolism of VLDL in adipocytes increase the accumulation of triglycerides in hepatocytes. The entry of proinflammatory cytokines from lysed fat tissue during insulin resistance stimulates hepatocytes to produce acute phase proteins (Wilcox, 2005; Reaven, 2004).

Insulin resistance can be related to disturbed health of cows in the peripartal period. For example, ketotic cows show significantly reduced insulin concentrations after glucose load (Djoković *et al.*, 2007, 2009, 2017). Another serious health problem in the peripartal period is fat infiltration of hepatocytes, a consequence of metabolic stress. Lipid accumulation in the liver is directly correlated with reduced insulin sensitivity and reduced insulin response to stimulation (Ohtsuka *et al.*, 2001; Oikawa and Oetzel, 2006). Release of inflammatory mediators such as tumor necrosis factor alpha (TNF- α) during sickness can interfere with insulin sensitivity in cattle (Kushibiki *et al.*, 2001).

Body condition is a major factor in the development of insulin resistance. Cows with greater body condition scores and obese cows develop significant insulin resistance during the peripartal period (Holtenius and Holtenius, 2007). This can be explained by the fact that obese cows have higher amounts of NEFA from fat in the period after calving, which correlates with TNF- α release.

Treatment with niacin caused dual changes in insulin resistance: decreased NEFA concentrations led to a decrease in insulin resistance (due to an increase in both insulin efficiency and the insulin sensitivity index), but increased concentrations of insulin and glucose possibly caused an increase in insulin resistance in dairy cows (due to the lower insulin sensitivity index and possibly lower antilipolytic effects of insulin) (Hristovska *et al.*, 2017).

Heat stress is an important factor affecting insulin sensitivity in dairy cows. Results (Majkić *et al.*, 2017) showed that: a) Cows exposed to heat-stress (high THI index) have lower milk production, higher concentrations of insulin and lower concentrations of glucose and NEFA; b) indices of insulin resistance-RQUICKI, insulin:glucose and insulin:NEFA are higher in cows under heat stress. The decrease in glucoses followed by an increased insulin level and increased insulin sensitivity indicate that glucose is transferred from the udder to other tissues, which can cause decreased milk production.

Measurement of insulin resistance

The euglycemic hyperinsulinemic clamp technique (gold standard test)

Several clinical research techniques have been developed to measure insulin resistance. The most widely used method is the euglycemic hyperinsulinemic clamp technique, which is generally considered to be the gold standard for measuring insulin sensitivity *in vivo* (Bergman *et al.*, 1989; Holtenius and Holtenius, 2007; Mashek *et al.*, 2001). With this technique, a primed-continuous infusion of insulin is administered to raise the plasma insulin concentration to a predetermined physiological or pharmacological level. The plasma glucose concentration is then measured at 5-minute intervals and a variable infusion of exogenous glucose is administered to maintain the plasma glucose concentration constant at the fasting level. Since the plasma glucose concentration remains unchanged, the amount of exogenous glucose infused must equal the amount of glucose utilized in response to the hyperinsulinemia and, thus, provides a direct measure of whole-body sensitivity to insulin. The insulin clamp is frequently combined with infusions of small amounts of stable or radioisotopically labeled glucose to measure hepatic glucose production or with labeled amino acids or fatty acids to measure protein or lipid metabolism. It also can be combined with indirect calorimetry to measure rates of glucose or lipid oxidation and non-oxidative glucose disposal, hemodynamic measures to assess the effects of insulin on vascular function or with imaging techniques to measure rates of glucose metabolism in specific tissues or organs.

The intravenous glucose tolerance test (FSIVGTT)

The other most widely used method for assessing insulin sensitivity is the frequently sampled intravenous glucose tolerance test (FSIVGTT), also known as

the minimal model technique (*Bergman et al., 1989; Holtenius and Holtenius, 2007; Djokovic et al., 2007, 2009, 2017*). With this procedure, a bolus of glucose, typically 300 mg/kg, is rapidly administered intravenously and plasma glucose and insulin levels are measured frequently for the next 3 hours. The resulting curves defined by the changes in plasma glucose and insulin are then fit using a non-linear modeling algorithm to derive indices reflecting the rate of change in plasma glucose level in response to the ambient insulin level (insulin sensitivity), the rate of change in glucose independent of the level of insulin (glucose effectiveness) and first and second phases of insulin secretion. Although this technique is somewhat simpler to perform than the insulin clamp, it does not provide a steady state of glucose metabolism in response to a known level of insulin and thus typically cannot be combined with the other techniques used to assess intermediary metabolism, substrate oxidation, hemodynamic measures or imaging techniques. Many other techniques for measuring insulin sensitivity also have been developed, including the insulin suppression test (*Bergman et al., 1989*), isolated organ or limb perfusion techniques and insulin measurements during an oral glucose tolerance test. Because the aforementioned techniques are labor intensive and not suited for large population-based studies or routine clinical use, several simpler measures have been proposed for these purposes (*Rabasa-Lhoret et al., 2003; Pires et al., 2007b, 2008; Alves-Nores et al., 2017*).

The homeostasis model assessment of insulin resistance (HOMA-IR)

The most widely used is the homeostasis model assessment of insulin resistance (HOMA-IR), which is calculated as [fasting plasma insulin level (in $\mu\text{U/ml}$) \times fasting plasma glucose level (in mmol/l)]/22.5 (*Holtenius and Holtenius, 2007; Balogh et al., 2008; Singh and Saxena, 2010; Cincovic 2017b*). A value of 1.00 is considered normal and higher values indicate progressively severe states of insulin resistance.

The quantitative insulin sensitivity check index (QUICKI)

A similar measure known as the quantitative insulin sensitivity check index (QUICKI) is used by some investigators and is calculated as $1/[\log \text{fasting plasma glucose (in mg/dL)} + \log \text{fasting plasma insulin (in } \mu\text{U/ml)}]$ (*Holtenius and Holtenius, 2007; Balogh et al., 2008; Cincovic et al., 2014, 2017a, 2017b; Djokovic et al., 2017*). Both of these measures are much more variable than either the insulin clamp or FSIVGTT, primarily due to the wide range of 'normal' values for fasting

plasma insulin. Also, they cannot be used in patients with diabetes where the normal homeostatic relationship between plasma glucose and insulin levels no longer exists and they have theoretical limitations based on the fact that they attempt to measure insulin sensitivity in the fasting state when the majority of glucose uptake is independent of insulin.

Revised quantitative insulin sensitivity index (RQUICKI)

The Revised Quantitative Insulin Sensitivity Check Index (RQUICKI) adapted from human medicine and used in cattle by *Holtenius and Holtenius (2007)* for the rapid estimation of insulin sensitivity was calculated in this study following the equation of $RQUICKI = 1/[\log(\text{conc.}\uparrow\text{ glucose}) + \log(\text{conc.}\uparrow\text{ insulin}) + \log(\text{conc.}\uparrow\text{ NEFA})]$. In general, a higher value of the index means higher insulin sensitivity. Hyperketonemia has been previously reported to interfere with insulin resistance. Depressed pancreatic insulin secretion following ivGTT and lower glucose and insulin clearance rates have been shown by several authors (*Djokovic et al., 2007, 2019*). Nowadays, in dairy cows, insulin resistance is evaluated by the value given by the RQUICKI formula $RQUICKI = 1/ [\log (\text{glucose mg/dL}) + \log (\text{insulin } \mu\text{U/mL}) + \log (\text{NEFA mmol/l})]$, (revised quantitative insulin sensitivity check index). This is a mathematical formula which is used for evaluating the homeostasis of energy balance which integrates the determination of glucose, insulin and NEFA concentrations. Characteristics of some tests, their usability, test performance problems, result interpretation problems and test development prospects are dealt with in the review paper of *Muniyappa et al. (2007)*. We have demonstrated that RQUICKI was not affected by the adaptations that normally occur in apparently healthy cows during the first months of lactation. However, RQUICKI was lower, reflecting disturbed insulin function, in obese cows. The results indicate that RQUICKI might be used to identify cows with disturbed insulin function. Our results have shown a correlation between HOMA, QUICKI and RQUICKI indices and metabolic profile parameters (*Cincovic et al., 2014, 2017a, 2017b; Djokovic et al., 2017*). However evidence of a relationship between RQUICKI and metabolic diseases in dairy cows is yet lacking. (*Rabasa-Lhoret et al., 2003; Balogh et al., 2008; Cincovic et al., 2014, 2017a, 2017b; Djokovic et al., 2017*).

RQUICKI-BHB - modified variant for RQUICKI

Therefore, we further modified the RQUICKI adding basal plasma concentration of β -hydroxybutyrate (BHB) into the equation, which then became $RQUICKI\ BHB = 1/[\log(\text{conc.}\uparrow 0\ \text{glucose}) + \log(\text{conc.}\uparrow 0\ \text{insulin}) + \log(\text{conc.}\uparrow 0\ \text{NEFA}) + \log(\text{conc.}\uparrow 0\ \text{BHB})]$. We expect that the inclusion of BHB will help to quickly, easily and more accurately assess insulin sensitivity. The revised quantitative insulin sensitivity check index and its modified variant (RQUICKI-BHB) seem equally able to estimate changes in insulin sensitivity. Both RQUICKI and RQUICKIBHB showed significant negative correlations with many of the glucose parameters of the ivGTT and with basal and mean 75-180 insulin concentrations, and were positively related to insulin clearance rate. Therefore, we infer that both indexes may be useful for the rapid estimation of insulin sensitivity in dairy cows (Balogh *et al.*, 2008; Cincovic *et al.*, 2014, 2017a, 2017b; Djokovic *et al.*, 2017). The correlation between dynamic and basal responses of NEFA, BHB, insulin, glucose and inorganic phosphorus is significantly dependent on RQUICKI-BHB index values in ketotic cows. RQUICKI index of insulin resistance is substantially dependent on NEFA values in early lactation as well as on insulin and glucose values in the dry period (Cincovic *et al.*, 2014, 2017a, 2017b; Djokovic *et al.*, 2017).

Conclusions

Insulin resistance is a state in which the biological effect of insulin is reduced when its concentration decreases or when a compensatory mechanism increases its concentration. Insulin resistance is characterized by reduced insulin response to glucose, i.e. decreased pancreatic beta cell function (insulin hyporesponsiveness) and/or reduced sensitivity of glucose to insulin (reduced intake of glucose by peripheral tissues under the influence of insulin – *eng.* insulin sensitivity). Methods for estimating insulin resistance include direct methods (hyperinsulinaemic-euglycaemic clamp, golden standard), indirect methods (intravenous glucose tolerance test) and surrogate methods (indices calculated from basal concentrations of insulin, glucose, NEFA and BHB, (HOMA, QUICKI, RQUICKI and RQUICKI- BHB). Surrogate indices show correlations with direct and indirect test results but they are inconsistent. Inconsistency occurs because the dependence of glucose concentrations on the degree of hepatic gluconeogenesis should be kept in mind when evaluating insulin resistance in ruminants. Therefore, the hyperinsulinaemic-euglycaemic clamp method is particularly suitable as it

excludes gluconeogenesis in hepatocytes from analysis. Our results have shown a correlation between HOMA, QUICKI and RQUICKI indices and metabolic profile parameters. The correlation between dynamic and basal responses of NEFA, BHB, insulin, glucose and inorganic phosphorus is significantly dependent on RQUICKI-BHB index values in ketotic cows. RQUICKI index of insulin resistance is substantially dependent on NEFA values in early lactation as well as on insulin and glucose values in the dry period.

Insulinska rezistencija kod mlečnih krava

Zoran Ilić, Radojica Đoković, Marko Cincović, Vladimir Kurćubić, Miloš Petrović, Milan P. Petrović, Violeta Caro Petrović

Rezime

Insulinska rezistencija je stanje u kome je smanjen biološki efekat insulina, u uslovima kada opada njegova koncentracija ili kada kompenzatorno može doći do povećanja njegove koncentracije. Insulinska rezistencija se karakteriše smanjenim odgovorom insulina na glukozu tj. smanjenom funkcijom beta ćelija pankreasa (eng. *insulin hyporesponsiveness*) i/ili smanjenom osetljivošću glukoze na insulin tj. smanjenim ulaskom glukoze u tkivo pod dejstvom insulina (eng. *insulin sensitivity*). Metode za merenje insulinske rezistencije mogu se podeliti na: direktne (hiperinsulinemijski euglikemijski klamp, zlatni standard), indirektne (intravenski glukoza tolerans test) i surogat metode (indeksi dobijeni iz bazalne koncentracije insulina, glukoze, NEFA i BHB - HOMA, QUICKI, RQUICKI i RQUICKI-BHB). Surogat indeksi pokazuju povezanost sa rezultatima iz direktnog i indirektnog testa, ali su rezultati nekonzistentni. Nekonzistentnost nastaje jer prilikom procene insulinske rezistencije kod preživara treba imati u vidu da je glikemija zavisna od stepena glukoneogeneze koja se odvija u jetri. Zbog toga je hiperinsulinemijski-euglikemijski klamp metod posebno kvalitetan jer u potpunosti isključuje glukoneogenezu u hepatocitima iz analize. Naši rezultati su pokazali da postoji korelacija HOMA, QUICKI i RQUICKI indeksa sa parametrima metaboličkog profila. Povezanost bazalnog i dinamičkog odgovora NEFA, BHB, insulina, glukoze i neorganskog fosfora značajno je determinisana vrednostima RQUICKI-BHB indeksa kod krava u ketozi. RQUICKI indeks insulinske rezistencije značajno je determinisan vrednostima NEFA u ranoj laktaciji i vrednostima insulina i glukoze u periodu zasušenja.

Ključne reči: mlečne krave, insulinska rezistencija, HOMA, QUICKI, RQUICKI, RQUICKI- BHB.

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THE INFLUENCE OF MASTITIS RISK ON RESPONSE TO HEAT STRESS IN DAIRY SIMMENTAL COWS

Goran Vučković¹, Mirna Gavran², Maja Gregić², Pero Mijić², Ranko Gantner², Marcela Šperanda², Vesna Gantner²

¹ Rinderunion Baden-Württemberg, Herberlingen, Germany

² Department for animal production and biotechnology, Faculty of agrobiotechnology Osijek, University of Josip Juraj Strossmayer in Osijek, Osijek, Croatia

Corresponding author: Vesna Gantner, vgantner@fazos.hr

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Abstract: Aiming determination of the influence of mastitis risk (defined accordingly to daily somatic cell count) on response to heat stress (defined as relationship between daily milk traits and microclimate parameters) in dairy Simmental cows over two million test-day records were analysed. The relationship between daily milk traits and microclimate parameters (ambient temperature, relative humidity, and THI) as well as variation in daily milk traits due to heat stress was tested for each mastitis risk class using PROC GLM/SAS. Based on the performed analysis, the negative effect of the increase of microclimate parameters on daily milk production in all cows was determined. Also, the highest decrease of daily production due to temperature and THI increase was determined in healthy cows, while high humidity had the highest effect on cows in high mastitis risk. Furthermore, the difference between the milk production in normal condition comparing to heat stress vary depending of THI threshold value and mastitis risk that is the highest decline in performance could be expected in cows in high mastitis risk in environment characterized by highly pronounced heat stress.

Key words: daily milk traits, dairy Simmentals, microclimate parameters, mastitis risk

Introduction

Today's milk production is characterized by high cattle productivity requirements and increasingly pronounced unfavourable (micro) climate conditions. Accordingly, to the *IPCC (2007)* changes in climate will significantly affect animal production worldwide. In support to stated, *Battisti and Naylor (2009)* forecasted that by year 2050, most of the world will be exposed to median temperatures in the summer that will be higher than the highest recorded temperatures. Furthermore, *Reiczigel et al. (2009)* in Hungary, as well as *Dunn et*

al. (2014) in UK indicated an increase of heat stress days per year. *Gauly et al.* (2013) emphasized that the heat stress of high productive dairy cows will cause growing concern in dairy production in Europe. *Segnalini et al.* (2013) pointed out the necessity of adequate adaptation strategies in order to decrease negative effects of climate change on domestic animals. *Hansen* (2013) stated that the high production makes cows more susceptible to heat stress meaning that heat stress will become, and already is, a problem in intensive dairy breeding systems regardless the climate changes. *Bohmanova* (2006) and *Collier et al.* (2006) determined that production level significantly alter the animal response to heat stress making high production animals more sensitive to heat stress than low production ones. The intensive genetic selection for high milk production resulted in changes in the thermoregulation physiology of dairy cattle (*Kadzere et al.*, 2002). Larger frames and larger gastrointestinal tracts in high production animals enable them to digest more feed, but also create more metabolic heat and reduces the animal's ability to regulate temperature heat stress environment meaning that increased milk yield, feed intake and metabolic heat the thermoneutrality to lower temperatures. Accordingly, to many studies, heat stress conditions in dairy cattle reduce dry matter intake, milk production (*West et al.* 1999; *Casa and Ravelo*, 2003; *Gantner et al.* 2011) as well as reproductive performances (*Bohmanova et al.* 2007; *Ravagnolo et al.* 2000). Furthermore, heat stress alters the milk composition, somatic cell counts (SCC) and prevalence of mastitis (*Bouraoui et al.* 2002; *Collier et al.* 2012; *Correa-Calderon et al.* 2004; *Gantner et al.* 2011, 2017; *Ravagnolo et al.* 2000; *St-Pierre et al.* 2003; *West* 2003; *Hammami et al.* 2013; *Smith et al.* 2013). Finally, heat stress induces considerable profit loss (*St-Pierre et al.*, 2003). The most common measure of heat stress in dairy cattle is temperature-humidity index (THI) that includes ambient temperature and relative humidity (*Kibler*, 1964). The THI threshold value at which heat stress affects milk production and feed intake vary, depending on study, from 68 to 72 (*Du Preez et al.*, 1990a, b; *Bouraoui et al.*, 2002; *Bernabucci et al.*, 2010; *Gantner et al.* 2011; *Collier and Hall*, 2012). *Vitali et al.* (2009) emphasised the increased risk of animals' death at THI = 80. Furthermore, the acclimatization to long-term stress results in proteomic changes indicated by expression of proteins related to inflammation (*Balfoussia et al.*, 2014). *Min et al.* (2016) concluded that long-term moderate heat stress may lead to an inflammatory response in dairy cows with significantly increased plasma TNF- α and IL-6, which present a pro-inflammatory factors. Since mastitis causes high level of stress and inflammatory changes in animals the aim of this research was to determine the influence of mastitis risk (defined accordingly to daily somatic cell count) on response to heat stress (defined as relationship between daily milk traits and microclimate parameters) in dairy Simmental cows.

Material and Methods

Over two million test-day records of dairy Simmental cows provided by the Croatian Agricultural Agency were used for statistical analysis. Test-day records were collected during the regular milk recording in the period from January 2005 to December 2012 in Croatia. Furthermore, milk recording was performed monthly accordingly to alternative milk recording method (AT4/BT4) that is at each recording, measuring and sampling of milk were performed during the evening or morning milkings. Additionally, at each milk recording, ambient temperature and relative humidity were recorded. Daily values of temperature-humidity index (THI) were calculated accordingly to Kibler equation (1964):

$$THI = 1.8 \times Ta - (1 - RH) \times (Ta - 14.3) + 32$$

Where Ta is average temperature in degrees Celsius and RH is relative humidity as a fraction of the unit. Test-day records with lactation stage in (< 5 days and > 500 days), age at first calving in (< 21 and > 36 months), missing parity, and missing or nonsense daily milk traits, Ta and RH value were deleted from the dataset. After logical control dataset consisted of 1,636,192 test-day records from 117,659 Simmentals reared on 10,599 farms. In accordance to mastitis risk that is somatic cell count (SCC), records were divided into three classes: NMS – healthy cows (SCC < 200,000); LMS – cows in low mastitis risk (SCC in 200,000 – 400,000); HMR – cows in high mastitis risk (SCC > 400,000). Variability of daily milk traits (daily milk yield; daily fat content; daily protein content; daily fat yield; daily protein yield) accordingly to mastitis risk is presented in Table 1.

Table 1 Basic statistical parameters (mean; SD) of daily milk traits accordingly to mastitis risk

Daily milk trait	Mastitis risk		
	NMR (SCC < 200,000)	NMR (SCC < 200,000)	NMR (SCC < 200,000)
Daily milk yield, kg	16.09±6.00	14.64±5.70	14.51±5.71
Daily fat content, %	4.13±0.88	4.23±0.89	4.26±0.94
Daily protein content, %	3.42±0.45	3.53±0.47	3.53±0.49
Daily fat yield, kg	0.66±0.27	0.61±0.26	0.61±0.26
Daily protein yield, kg	0.53±0.19	0.51±0.18	0.50±0.19

The relationship between daily milk traits (yields and contents) and microclimate parameters (T, RH, and THI) was analysed using PROC GLM procedure in SAS (SAS Institute Inc., 2000) accordingly to following model 1:

$$y_{ijklmn} = \mu + b_1(d_i / 305) + b_2(d_i / 305)^2 + b_3 \ln(305 / d_i) + b_4 \ln^2(305 / d_i) + S_j + A_k + R_l + x_{ijklm} + e_{ijklm} \quad (1)$$

While the variation in daily milk traits (yields and contents) due to heat stress was tested by least square analyses of variance for each given THI value (65, 70 and 75) separately for each mastitis risk (NMR, LMR, HMR) class using PROC GLM / SAS accordingly to following model 2:

$$y_{ijklm} = \mu + b_1(d_i / 305) + b_2(d_i / 305)^2 + b_3 \ln(305 / d_i) + b_4 \ln^2(305 / d_i) + S_j + A_k + R_l + T_m + x_{ijklm} + e_{ijklm} \quad (2)$$

Where y_{ijklm} = estimated daily milk trait;

μ = intercept;

b_1, b_2, b_3, b_4 = regression coefficients;

d_i = days in milk ($i = 5$ to 500 day);

S_j = fixed effect of recording season class j ($j = 1/2005$ to 12/2012);

A_k = fixed effect of age at calving class k ($k = 21$ to 36 month);

R_l = fixed effect of breeding region l ($l =$ Eastern, Central, and Mediterranean Croatia);

x_{ijklm} = fixed effect of microclimate parameter as linear regression – model 1;

T_m = fixed effect of THI class ($m = 0$ (*normal condition – values under the given threshold*) or $m = 1$ (*heat stress condition – values equal and above the given threshold*)) – model 2;

e_{ijklm} = residual.

The significance of the differences between the THI classes was tested by t-test.

Results

The negative relationship between daily milk traits and microclimate parameters was determined in all cows regardless the mastitis risk (Table 2). The highest decrease of daily milk yield due to temperature increase was determined in healthy cows in amount of 36.5 g of milk/°C. The increase of relative humidity had the highest negative effect on daily production of cows in high mastitis risk, while the highest decline in daily production due to THI increase was determined in healthy cows (24.3 g of milk/THI unit). Furthermore, the increase of temperature and relative humidity in the barns resulted in slightly decrease of daily fat/protein

contents/yields, with similar amount of decrease in all cows regardless the risk of mastitis.

Table 2 The coefficients of linear regression between daily milk traits and microclimate parameters accordingly to mastitis risk

Daily milk trait	Microclimate parameter / Mastitis risk								
	Ambient temperature, T, °C			Relative humidity, RH, %			THI		
	NMR	LMS	HMS	NMR	LMS	HMS	NMR	LMS	HMS
DMY, kg	- 0.0365	- 0.0265	- 0.0246	- 0.0043	-0.0041	-0.0057	- 0.0243	- 0.0184	- 0.0169
DFC, %	- 0.0069	- 0.0067	- 0.0066	- 0.0006	-0.0004	-0.0003	- 0.0046	- 0.0045	- 0.0044
DPC, %	- 0.0033	- 0.0036	- 0.0036	- 0.0001	0.00001	0.00007	- 0.0022	- 0.0023	- 0.0024
DFY, kg	- 0.0026	- 0.0022	- 0.0020	- 0.0003	-0.0002	-0.0003	- 0.0018	- 0.0015	- 0.0014
DPY, kg	- 0.0018	- 0.0015	- 0.0014	- 0.0001	-0.0001	-0.0002	- 0.0012	- 0.0010	- 0.0009

DMY – daily milk yield; DCF – daily fat content; DPC – daily protein content; DFY – daily fat yield; DPY – daily protein yield; all coefficients of linear regression were statistically highly significant ($p < 0.001$)

Statistically highly significant ($p < 0.001$) decrease in daily milk traits due to heat stress condition when THI threshold was set at 65 was determined in cows in all mastitis risk classes (Table 3). The highest difference between the daily milk yield produced in normal condition comparing to heat stress condition in amount of 201 g of milk/day was determined in healthy cows, while the lowest difference in amount of 62 g of milk/day was determined in cows in high mastitis risk. Furthermore, the amount of decrease in daily fat/protein content/yield was similar in all cows.

Table 3 LSmeans of daily milk traits regarding the heat stress (HS-0/HS-1) when threshold THI = 65 accordingly to mastitis risk

Mastitis risk / daily milk trait	NMR		LMR		HMR	
	HS-0	HS-1	HS-0	HS-1	HS-0	HS-1
DMY, kg	14.261 ^A	14.060 ^B	14.258 ^A	14.179 ^B	14.105 ^A	14.043 ^B
DFC, %	4.071 ^A	3.995 ^B	4.245 ^A	4.170 ^B	4.303 ^A	4.224 ^B
DPC, %	3.361 ^A	3.324 ^B	3.534 ^A	3.493 ^B	3.532 ^A	3.489 ^B
DFY, kg	0.574 ^A	0.554 ^B	0.597 ^A	0.583 ^B	0.599 ^A	0.586 ^B
DPY, kg	0.474 ^A	0.462 ^B	0.496 ^A	0.487 ^B	0.490 ^A	0.482 ^B

DMY – daily milk yield; DCF – daily fat content; DPC – daily protein content; DFY – daily fat yield; DPY – daily protein yield; LSmeans within the same row and mastitis risk class marked with different letters (A, B) differ statistically highly significant ($p < 0.001$); HS-0: normal condition, HS-1: heat stress condition

Simmentals response to heat stress regarding the mastitis risk at threshold THI = 70 is presented in Table 4. Statistically highly significant ($p < 0.001$) decrease in daily milk yields and contents due to heat stress condition was determined in all animals. The difference between the daily milk yield produced in normal condition comparing to heat stress condition amounted 175 g, 131 g, and 118 g of milk/day in healthy, and in cows in low and high mastitis risk, respectively. Comparing with lower THI threshold (THI = 65), the difference in daily milk yield at THI = 70 was almost 50% lower in healthy cows, and almost three times higher in cows in high mastitis risk. The difference in daily fat/protein content/yield between the normal and heat stress conditions was similar in all mastitis risk classes.

Table 4 LSmeans of daily milk traits regarding the heat stress (HS-0/HS-1) when threshold THI = 70 accordingly to mastitis risk

Mastitis risk / daily milk trait	NMR		LMR		HMR	
	HS-0	HS-1	HS-0	HS-1	HS-0	HS-1
DMY, kg	14.229 ^A	14.054 ^B	14.257 ^A	14.126 ^B	14.106 ^A	13.988 ^B
DFC, %	4.059 ^A	3.993 ^B	4.232 ^A	4.174 ^B	4.291 ^A	4.220 ^B
DPC, %	3.356 ^A	3.321 ^B	3.528 ^A	3.493 ^B	3.525 ^A	3.489 ^B
DFY, kg	0.571 ^A	0.554 ^B	0.594 ^A	0.582 ^B	0.597 ^A	0.583 ^B
DPY, kg	0.472 ^A	0.461 ^B	0.495 ^A	0.486 ^B	0.489 ^A	0.480 ^B

DMY – daily milk yield; DCF – daily fat content; DPC – daily protein content; DFY – daily fat yield; DPY – daily protein yield; LSmeans within the same row and mastitis risk class marked with different letters (A, B) differ statistically highly significant ($p < 0.001$); HS-0: normal condition, HS-1: heat stress condition

The highest difference between the daily milk yield produced in normal comparing to heat stress condition in cows in mastitis risk was determined at threshold THI = 75 (Table 5). The difference amounted 190 g of milk/day cows in low mastitis risk, while in cows in high mastitis risk the difference amounted 185 g of milk/day was determined. Healthy cows had lowest decrease in daily milk yield due to heat stress at THI = 75. Similarly like at lower THI threshold values (65 and 70), the amount of decrease in daily fat/protein content/yield at THI = 75 was similar in all animals regardless the mastitis risk.

Table 5 LSmeans of daily milk traits regarding the heat stress (HS-0/HS-1) when threshold THI = 75 accordingly to mastitis risk

Mastitis risk / daily milk trait	NMR		LMR		HMR	
	HS-0	HS-1	HS-0	HS-1	HS-0	HS-1
DMY, kg	14.214 ^A	14.027 ^B	14.250 ^A	14.060 ^B	14.101 ^A	13.916 ^B
DFC, %	4.052 ^A	3.996 ^B	4.226 ^A	4.187 ^B	4.284 ^A	4.222 ^B
DPC, %	3.352 ^A	3.323 ^B	3.524 ^A	3.498 ^B	3.531 ^A	3.492 ^B
DFY, kg	0.569 ^A	0.554 ^B	0.594 ^A	0.581 ^B	0.596 ^A	0.580 ^B
DPY, kg	0.471 ^A	0.461 ^B	0.494 ^A	0.484 ^B	0.489 ^A	0.478 ^B

DMY – daily milk yield; DCF – daily fat content; DPC – daily protein content; DFY – daily fat yield; DPY – daily protein yield; Lsmeans within the same row and mastitis risk class marked with different letters (A, B) differ statistically highly significant ($p < 0.001$); HS-0: normal condition, HS-1: heat stress condition

Discussion

The negative effect of microclimate parameters increase on daily milk production was determined in all animals regardless the mastitis risk. The increase of ambient temperature for one degree, in healthy cows, induces decrease of 36.5 g of milk/day. Furthermore, the negative effect of high relative humidity in the barns was most pronounced in cows in high mastitis risk, while the increase of THI had the strongest effect on healthy cows with the drop of 24.3 g of milk per THI unit. Highly productive dairy cattle lose their ability to regulate body temperature at an air temperature of only 25 to 29°C. For instance, *Berman (2005)* determined that increase in daily production from 35 to 45 kg results in a higher sensitivity to thermal stress and reduces the threshold temperature for intermediate heat stress by 5°C. *Gantner et al. (2011)* indicated that the heat stress condition may occur even in periods with lower temperatures when problems could be caused by high relative humidity. The negative effect of high humidity on milk production was also confirmed by *Bianca (1965)*. She determined that at 29°C and 40% RH milk yield of Holstein, Jersey and Brown Swiss cows produced 97, 93, and 98% of normal production, but when relative humidity increased to 90% milk production dropped to 69, 75, and 83% of normal level. In dairy cows reared in South Africa, *Du Preez et al. (1990a)* determined the decrease of milk yield at THI higher than 72 (22°C at 100% RH, 25°C at 50% RH, or 28°C at 20% RH). Significant decrease in milk production, in amount of 6% (9%) depending on the region, during the warmer months in Argentina was also determined by *Casa and Ravelo (2003)*. Similarly, to results of this research, *Bouraoui et al. (2002)* reported decrease of 0.41 kg of milk/day for each point increase of THI above 69. The threshold value of THI depend on a many effects, for instance, lactation stage, parity, level of milk production, breed, breeding region, individual susceptibility to heat stress, etc. (*Kadzere et al. 2002, Bohmanova 2006, Collier et al. 2006, Hansen 2013, Gantner et al. 2017*). For instance, *Bouraoui et al. (2002)* put the threshold on 65-69, *Bernabucci et al. (2010)* as well as *Collier and Hall (2012)* on 68, *Du Preez et al. (1990a, b)* on 72, while *Bohmanova et al. (2007)* depending on region defined threshold THI value 72 in Georgia, and 74 in Arizona. *Lambertz et al. (2014)* pointed out that the difference in defined threshold values could be due to better adapted cows, farm management or housing. In this research, the difference in response to heat stress duo to risk of mastitis (and accompanying stress and inflammatory changes in animals) was analysed. Statistically highly significant

($p < 0.001$) decrease in daily milk traits due to heat stress condition at each set THI threshold value (65, 70, and 75) was determined in all cows in all mastitis risk classes. Furthermore, the highest difference between the daily milk yields produced in normal condition comparing to heat stress condition at the lowest THI (65) was determined in healthy cows, while at the highest THI (75) the highest difference in daily milk yield was determined in cows in high mastitis risk. Obtained results indicate variability in response to heat stress due to risk of mastitis that is different reaction of animals' organism to new stressor.

Conclusion

Based on the conducted research, the negative effect of microclimate parameters increase on daily milk production in all cows could be pointed out. Also, highest decrease of daily production due to temperature and THI increase was determined in healthy cows, while high humidity had the highest effect on cows in high mastitis risk. Furthermore, the difference between the milk production in normal condition in comparison to heat stress vary depending of THI threshold value and mastitis risk that is the highest decline in performance could be expected in cows in high mastitis risk in environment characterized by highly pronounced heat stress (overlapping of two stressors).

Uticaj rizika od mastitisa na reakciju na toplotni stres kod krava simentalke rase

Goran Vučković, Mirna Gavran, Maja Gregić, Pero Mijić, Ranko Gantner, Marcela Šperanda, Vesna Gantner

Rezime

U cilju određivanja uticaja rizika od mastitisa (definisan u skladu sa dnevnim brojem somatskih ćelija) na reakciju na toplotni stres (definisan kao odnos između dnevnih osobina mleka i mikroklimatskih parametara) kod mlečnih krava simentalke rase analizirano je preko dva miliona podataka dobijenih na dan kontrole mlečnosti. Odnos između osobina dnevnog mleka i parametara mikroklimatske (temperatura okoline, relativna vlažnost i THI) kao i promene u dnevnim mlečnim osobinama usled toplotnog stresa analiziran je za svaki razred rizika od mastitisa korišćenjem PROC GLM / SAS. Na osnovu izvršene analize utvrđen je negativan uticaj povećanja mikroklimatskih parametara na dnevnu proizvodnju mleka kod svih krava. Takođe, najveći pad dnevne proizvodnje usled porasta temperature i THI utvrđen je kod zdravih krava, dok je visoka vlažnost

vazduha imala najveći uticaj na krave u visokom riziku od mastitisa. Štaviše, razlika između proizvodnje mleka u normalnom stanju u poređenju sa toplotnim stresom varira u zavisnosti od vrednosti THI praga i rizika od mastitisa koji je najveći pad performansi, može se očekivati kod krava visokog rizika od mastitisa u okruženju koje karakteriše izrazito izražen toplotni stres.

Ključne reči: dnevne osobine mleka, mlečna grla simentalске rase, parametri mikrokline, rizik od mastitisa

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EFFECT OF NATURAL STRONGYLUS SPP. INFECTIONS ON SYNOVIAL FLUID CONSTITUENTS IN DONKEYS

Mahmoud R. Abd Ellah¹, Ghada I. Soliman², Mohamed A.H. Abd Elhakeim³, Hanan K. Elsayed¹

¹Department of Animal Medicine, Faculty of Veterinary Medicine, Assiut University, Egypt.

²Postgraduate, Department of Animal Medicine Assiut Governorate, Egypt

³Department of Animal Surgery, Anesthesiology and Radiology, Faculty of Veterinary Medicine, Assiut University, Egypt.

Corresponding author: Mahmoud R. Abd Ellah, mrushdi@aun.edu.eg

Original scientific paper

Abstract: The present study was undertaken to investigate the effect of *Strongylus* spp. infection on synovial fluid constituents in donkeys. A total number of 23 donkeys constituted the material of this investigation. Biochemical constituents were measured in both synovial and serum samples. The results revealed no significant changes in synovial total white blood cells count in all donkeys infected with parasites in comparison to control. Serum total proteins and globulins were significantly decreased in case of severe *Strongylus* infection. Hypoglycemia were observed in case of severe infections with *Strongylus* spp. It could be concluded that, *Strongylus* spp. infection in donkeys results in significant biochemical changes in serum and synovial fluid, which may affect the performance of donkeys.

Keywords: Donkey, synovial fluids, parasites, *Strongylus* spp.

Introduction

Donkeys are still well deserving of the name (beasts of burden) they have a prominent position in the agricultural system of many developing countries (Pearson *et al.*, 1999). In underdeveloped countries, donkeys are used principally as draught or pack animals. In villages, donkeys are used for carriage purpose and a saddle animal (Uslu and Guclu, 2007).

Helminthes parasites, particularly Strongyle nematodes are the common inhabitants of the gastro-intestinal tract of equines and can cause infections with

clinical signs from ill-thrift to sudden death (*Umur and Acici, 2009*). Internal parasites cause significant clinico-pathological changes in donkeys (*Lewa et al., 1999*). Some parasitic infections even prove fatal if control measures are neglected (*Hayat et al., 1987*).

Studies of mammalian synovial fluid have found considerable similarities among species, although notable differences do exist. The majority of investigative work determining the composition of synovial fluid had been performed on equine synovia because large quantities of it are the available. The major difference between synovial fluid and other body fluids derived from plasma is the high content of hyaluronic acid (hyaluronan) in synovial fluid (*Lipowitz, 1985*).

Several studies were performed on human that declared the presence of a type of arthritis called reactive arthritis in cases that had parasitic infection with *Strongyloides stercoralis* (*Akoglu, 1984*) and *Taenia saginata* (*Bocanegra et al., 1981*), and with protozoa (*Tejera et al., 2012*). According to the authors' knowledge, there is no single record about studying the effect of parasitic infections on the constituents of synovial fluids in equines, which constituted the aim of this study.

Materials and Methods

A total number of 23 male and female donkeys constituted the material of this investigation. Their ages were varied from one to five years old. Donkeys were examined at the Veterinary Teaching Hospital, Assiut University, Egypt.

Animals were inspected for presence of any abnormal clinical signs and then blood, fecal and synovial fluid samples were collected. The general body conditions of the investigated animals were varied from good to emaciated. Animals with apparent lesions in skin were excluded from the study.

Two types of blood samples (whole blood and serum samples) were collected from all examined animals; Whole Blood samples were collected from the jugular vein over the inner side of Vacutainer tubes containing Ethylene diamine tetra-acetic acid (EDTA) as anticoagulant and used for white blood cells count according to *Coles (1986)*. Blood samples for separation of serum were collected in plain Vacutainer tubes (containing no anticoagulant) from each animal under the study. Serum samples were harvested according to *Coles (1986)*.

Fecal samples were collected from the rectum in clean and dry plastic cups and analyzed directly after collection using direct smear, sedimentation and floatation techniques, fecal egg count was performed using Mc Master Technique according to *Soulsby (1982)*.

The synovial fluid samples were collected from the carpal joint (radiocarpal and carpo-metacarpal joints) using sterile syringe and needle (22 gauge, 2 inch) under aseptic conditions. The carpal joints were examined before aspiration of the synovia. Which were free from any signs of inflammation such as swelling, hotness and discharges. The passive movement (extension and flexion) did not show any signs of pain or ankylosis. The synovia was aspirated when the animal in a standing position (*Reichman and Waddell, 2004*). The aspirated synovial samples were divided into two parts; one part was stored at 4 °C for 2 days to test clot formation and the other part was collected in Vacutainer tube containing heparin and mixed properly to prevent coagulation and used for physical, microscopical and biochemical analysis. Synovial fluid was examined physically, chemically and microscopically according to *Chauhan and Chandra (2007)*.

Biochemical constituents were measured in serum and synovial fluid samples, which included total protein, albumin, globulins, calcium, phosphorus, magnesium, glucose, gamma glutamyl transferase (GGT) and alkaline phosphatase (ALP) by using commercial kits supplied by Spectrum (Spectrum Diagnostics, Cairo, Egypt), and by means of UV spectrophotometer (Optizen 3220 UV, Mecasys Co. Ltd, Korea).

Statistical Analyses

Statistical analysis of the obtained data was done by using statistical package for the social sciences for windows (SPSS, version 16.0) according to *Borenstein et al. (1997)*. Data were presented as mean and standard deviation. Data from infected donkeys were compared with control group using one-way ANOVA. Statistically significant differences were determined at $P \leq 0.01$ (Highly significant) and $P < 0.05$ (Significant).

Results and Discussion

Donkey's subjected to study (n=23) were classified according to intestinal parasitic infection into two major groups: *Strongylus* spp. (n.=23), and control (n.=6) groups. *Strongylus* spp. group was re-classified according to the fecal egg count into; mild (0-500 epg), moderate (500-1000 epg) and severe (>1000 epg) infection groups (*Soulsby, 1982*).

In this study, synovial fluids from donkeys infected with *Strongylus* spp. parasites were colorless, transparent, and viscous. No coagulation was detected in synovial fluid stored at 4°C for 2 days. Results for the mucin test were the same in

different groups and appeared as a tight ropy clump in a clear solution. Mucin clot quality is a representative indication of the viscous property and quality of hyaluronic acid present in synovial fluid (McIlwraith, 1996). In our study, mucin clot quality test of intestinal parasites infected donkeys revealed normal viscosity, which indicated absence of inflammation and confirmed the results of physical examination of the synovial fluids. In the articular disease process, reduced polymerization of the hyaluronic acid molecule result in clot of poor quality and variable degree of flocculation appear in a cloudy solution (Palmer and AL, 1994; Smith *et al.*, 2002).

No significant changes were observed in synovial total WBCs count and in number of WBCs/HPF in all donkeys infected with parasites in comparison to control (Table 1). Total blood WBCs count showed no significant changes in all groups of donkeys infected with parasites compared to the control group, these findings are in accordance with the findings of Abd-El-Salam (1998). Total synovial WBCs count in all groups was lower than that reported by Abd Ellah *et al.* (2012), who found that WBCs count in synovial fluid from normal donkeys was $127.60 \pm 42.6/\text{mm}^3$. Normal synovial WBCs count in studied groups indicated absence of inflammatory changes in the joint, which is supported by normal physical characters of the synovial fluids.

Table 1. Effect of different degrees of *Strongylus* spp. infestation on blood and synovial WBCs in donkeys.

		<i>Strongylus</i> spp. infestation		
	Control (n.=6)	Mild (n.=7)	Moderate (n.=13)	Severe (n.=3)
Blood WBCs ($10^3/\text{mm}^3$)	11.63 ± 1.56	10.27 ± 2.35	12.02 ± 1.98	11.88 ± 2.26
Synovial WBCs (mm^3)	29.83 ± 7.27	24.00 ± 3.51	27.38 ± 5.97	23.66 ± 3.21
WBCs/HPF	2.00 ± 1.26	4.57 ± 1.27	4.00 ± 1.22	5.00 ± 1.73

Data were expressed as Mean \pm SD, ; WBCs: White blood cells; Mild: Mild *Strongylus* spp. infestation; Moderate: Moderate infestation with *Strongylus* spp. ; Severe: Severe *Strongylus* spp. infestation

Total protein concentration in serum is a common measurement in clinical laboratory diagnosis. The protein content of normal synovia has been determined for the hock joint by Kersjes (1963) and van Pelt *et al.* (1971). These authors found concentrations of 1.0-1.8 g/dl. Electrophoresis of synovial protein (Kersjes, 1963) indicated that the fluid contained 40-43 percent albumin and 57-60% globulins. Changes in the levels of plasma proteins may result from alteration in synthesis,

catabolism or from protein losses. In this study, serum total protein, albumin and globulin levels in control donkeys were almost the same as reported by *Ju et al. (1993)*. In the present study, the significant decreases ($P<0.01$) in serum total proteins and globulins in case of severe *Strongylus* infection (Table 2) agreed with that reported by *Abd-El-Salam (1998)*, who reported hypoproteinemia and hypoglobulinemia in heavy parasitic infection. Furthermore, *Smets et al. (1999)* found hypoproteinemia and hypoalbuminemia in horses infected with intestinal parasites. *Esmat et al. (1997)* found significant decreases in serum glucose, total serum protein, albumin and globulin in intestinal nematode infections of horses.

Hypoalbuminemia of the present study, corroborate well with findings reported by several workers including, *Jasko and Roth (1984)*; *Smets et al. (1999)*; *Lyons et al. (2000)*; *Peregrine et al. (2006)*; *Corning (2009)* and *Bodecek et al. (2010)*. They also recorded hypoalbuminemia in intestinal parasites infected horses. Hypoalbuminemia in the present study may be attributed to malnutrition that associates heavy parasitic infection (*Radostits et al. 2007*).

The obtained results revealed significant hypomagnesemia in serum of *Strongyle* sp. infected donkeys, which may be attributable to malabsorption of magnesium from the intestine as suggested by *Parsani et al. (2011)*. On the other hand, synovial calcium and phosphorus levels were significantly increased in severely infected group, which may be caused by the insignificant increase of both elements in serum.

Table 2. Effect of different degrees of *Strongylus* spp. infestation on serum biochemical constituents in donkeys.

	Control (n.=6)	<i>Strongylus</i> spp. infestation		
		Mild (n.=7)	Moderate (n.=13)	Severe (n.=3)
Total proteins (g/dl)	7.27±0.62	6.25±1.38	7.01±1.55	5.05±.45**
Albumin (g/dl)	2.60±0.07	2.20±0.42	2.31±1.18	2.30±0.40
Globulins (g/dl)	4.67±0.56	4.05±1.78	4.69±1.76	2.75±.85**
Calcium (mg/dl)	8.23±0.89	9.27±2.68	9.08±2.66	10.93±4.01
Phosphorus (mg/dl)	4.43±1.42	4.46±0.95	4.55±1.51	4.20±1.15
Magnesium (mg/dl)	2.87±0.36	2.30±0.435*	2.18±0.59**	1.92±0.14**
Glucose (mg/dl)	80.64±3.12	75.82±19.74	69.94±22.12	57.47±6.25**
GGT (U/l)	16.11±3.91	11.93±4.45	11.65±6.09	8.46±6.11
ALP (U/l)	137.77±46.92	170.84±92.65	155.24±51.31	154.96±38.66

Data were expressed as Mean ± SD, Mild: Mild *Strongylus* spp. infestation; Moderate: Moderate infestation with *Strongylus* spp.; Severe: Severe *Strongylus* spp. infestation

Glucose content tends to be similar in different joints, resembling the levels in serum (*van Pelt et al., 1971*). In this study, glucose levels in synovial fluid (85.32 ± 14.07 mg/dl) and serum (80.64 ± 3.12 mg/dl) were somewhat similar in donkeys that belonged to the control group (Table 3). In this study, blood glucose levels were within the normal range in healthy horses (Kahn and Line, 2011). Furthermore, the glucose concentration was higher in synovia than in blood, which disagree with the findings of *Kersjes (1963)* and agreed with *van Pelt et al. (1971)*, who found higher glucose concentrations in synovia. In the present investigation, reduction in serum and synovial glucose level in donkeys infected with parasites might be due to the fact that adult worms thrive on carbohydrate available in GI tract, consequently, depleting the host of the required glucose. It has also been reported that parasite count is inversely proportional to glucose concentration (*Jatkar and Singh, 1974*). Glucose malabsorption was probably due to derangement in the transfer capacity of the absorptive epithelium and an increase in the metabolism of the infected intestine (*Scofield, 1974*).

Table 3. Effect of different degrees of *Strongylus* spp. infestation on synovial biochemical constituents in donkeys.

	Control (n.=6)	<i>Strongylus</i> spp. infestation		
		Mild (n.=7)	Moderate (n.=13)	Severe (n.=3)
Total proteins (g/dl)	2.51 ± 0.61	2.11 ± 0.43	$2.32 \pm .86$	1.98 ± 1.04
Albumin (g/dl)	0.84 ± 0.50	$.93 \pm .59$	$.85 \pm .27$	$1.23 \pm .81$
Globulins (g/dl)	1.67 ± 1.05	$1.18 \pm .79$	$1.46 \pm .83$	$.75 \pm .62$
Calcium (mg/dl)	5.67 ± 1.23	7.10 ± 2.22	$7.57 \pm 1.95^*$	$7.85 \pm 1.26^*$
Phosphorus (mg/dl)	$3.69 \pm .24$	4.04 ± 1.19	4.69 ± 2.19	$6.60 \pm 2.74^*$
Magnesium (mg/dl)	$2.43 \pm .72$	$2.11 \pm .62$	$2.01 \pm .37$	$2.73 \pm .25$
Glucose (mg/dl)	85.32 ± 14.07	69.07 ± 21.16	$57.61 \pm 11.08^{**}$	$55.09 \pm 8.47^{**}$
GGT (U/l)	$7.09 \pm .54$	$4.02 \pm 1.47^{**}$	$4.10 \pm 1.56^{**}$	$4.40 \pm .00^{**}$
ALP (U/l)	26.94 ± 6.82	$73.17 \pm 44.83^*$	$49.51 \pm 17.32^{**}$	$64.66 \pm 14.91^{**}$

Data were expressed as Mean \pm SD, Mild: Mild *Strongylus* spp. infestation; Moderate: Moderate infestation with *Strongylus* spp.; Severe: Severe *Strongylus* spp. infestation

Enzyme profiles are one of main biochemical parameters used in equine medicine to assess muscle and liver functions. While serum level of GGT is usually measured to evaluate liver function (*Kaneko et al., 1997*), ALP is released from variety of sources including liver, intestine and bone. In the present study, synovial GGT activity was significantly decreased ($P < 0.01$) in case of infection with *Strongylus*. On the other hand, a significant increase in synovial ALP activity was observed in case of mild ($P < 0.05$), moderate ($P < 0.01$) and severe ($P < 0.01$)

infection with *Strongylus*. As in the present study, van Pelt *et al.* (1971) found far higher activities of the enzymes ALP in serum than in synovia. In this study, the levels of all measured blood variables were higher in blood than in synovial fluid, this because synovia derives its constituents from blood plasma (Liberg *et al.*, 1977).

Conclusion

Serum total proteins and globulins are decreased in case of severe *Strongylus* infection. Serum magnesium level is decreased in case of mild, moderate and severe *Strongylus* infection. Significant hypoglycemia accompanies severe infection with *Strongylus* spp. parasites in donkeys. Low level of synovial glucose accompanies moderate and severe *Strongylus* infection.

Uticaj prirodne infekcije *Strongylus* spp. na sastojke sinovijalne tečnosti kod magaraca

Mahmoud R. Abd Ellah, Ghada I. Soliman, Mohamed A.H. Abd Elhakeim, Hanan K. Elsayed

Rezime

Ova studija je preduzeta da bi se istražilo dejstvo *Strongylus* spp. infekcije na sastojke sinovijalne tečnosti u magaraca. Materijal ove istrage bio je ukupno 23 magaraca. Biohemijski sastojci su mereni u uzorcima sinovijalne kiseline i seruma. Rezultati nisu pokazali značajne promene u ukupnom broju sinovijalnih belih krvnih zrnaca kod svih magaraca zaraženih parazitima u odnosu na kontrolu. Ukupni proteini i globulini u serumu značajno su smanjeni u slučaju teške infekcije *Strongylus* spp. Hipoglikemija je primećena u slučaju teških infekcija *Strongylus* spp. Moglo bi se zaključiti da je, *Strongylus* spp. infekcija kod magaraca rezultira značajnim biohemijskim promenama u serumu i sinovijalnoj tečnosti, što može uticati na njihove performanse.

Ključne reči: magarac, sinovijalna tečnost, paraziti, *Strongylus* spp.

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SURVIVAL OF SALMONELLA IN PIG CARCASSES IN SLAUGHTERHOUSES

Jasna M. Kureljušić¹, Alesandra Tasić¹, Jadranka Žutić¹, Branislav Kureljušić¹, Ljiljana Spalević¹, Suzana Vidaković², Dragana Ljubojević²

¹Scientific Veterinary Institute of Serbia, Belgrade, Vojvode Tozebr 14, Serbia

²Scientific Veterinary Institute "Novi Sad", Rumenački put 20, Novi Sad

Corresponding author: Jasna Kureljušić, jasnakureljusic@yahoo.com

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Abstract: *Salmonella* has been widely recognized as the most important zoonotic pathogen with health and economic impact on animals and humans. It is one of the major bacterial causes of gastroenteritis worldwide. The microorganism may also be transmitted through a direct contact with infected animals or faecal-contaminated environments and humans. The most common symptom of non-typhoidal *Salmonella* infection is acute gastroenteritis. The objective of this experiment was to determine the status of *Salmonella* on pig carcasses at a slaughterhouse. For *Salmonella* detection, samples were analysed according to ISO 6579:2008 and positive samples were serotyped by Kauffman-White-Le-Minor technique. The presence of *Salmonella* spp. after stunning was determined in 41% of the carcasses (100 carcasses were examined) while after processing, *Salmonella* was confirmed in 2% of the tested carcasses. Detection of *Salmonella* after stunning was within a range of 0 to 90%, during ten weeks of sampling. After processing, *Salmonella* was isolated only in two carcasses both originating from the third week of the study.

Key words: *Salmonella* spp., pig carcass, slaughterhouses

Introduction

Salmonella genus is classified in the family of Enterobacteriaceae, whose members are non-encapsulated, gram-negative bacilli. Almost all *Salmonellas* are motile since they have peritrichous flagella, with the exception of *S. Gallinarum* and *S. Pullorum*, (Tindall *et al.*, 2005). The organism is 0.7-1.5 µm wide and 2.0-5.0 µm long. *Salmonella* is facultative anaerobic bacteria and uses citrate as a sole carbon source (Meneses, 2010). The organism also produces hydrogen sulphide gas on triple-sugar iron agar and on decarboxylate lysine and ornithine test reduces nitrates to nitrites. *Salmonella* is negative on urease and indole test (Nikaido *et al.*, 2011).

Salmonella growth rate is dependent on several factors including temperature, pH, water activity and nutrients (Meneses, 2010). Salmonella grows at temperatures between 10 and 49 °C, with an optimum of approximately 37 °C (Zeirak et al., 2012). At temperatures between 0 and 5 °C, the organisms remain viable even though there is no growth (Zeraik et al., 2012). Under freezing conditions and long-term frozen storage, there is a marked reduction in the number of Salmonellas, however not all of them are destroyed (Shah et al., 2013). Salmonella is inactivated when exposed to temperatures of 55 °C for one hour or 60 °C for 15 to 20 minutes (Aljarallah et al., 2007). Salmonella is eliminated during the cooking of food when the internal temperature of the food reaches 74 to 77 °C. Nevertheless, household-cooking procedures used for eggs and egg-containing foods are frequently insufficient to ensure a safe meal (Coetzer and Tustin, 2004).

Salmonella is one of the major bacterial causes of gastroenteritis worldwide (EFSA, 2014). The organism may also be transmitted through a direct contact with infected animals or faecal-contaminated environments and humans. The most common symptom of non-typhoidal Salmonella infection is acute gastroenteritis. Onset of intestinal salmonellosis occurs usually between a few hours and three to four days following ingestion of the infectious agent (Santana et al., 2012). Human salmonellosis is usually characterized by acute fever, abdominal pain, nausea, and sometimes vomiting (EFSA, 2014). Symptoms are often mild and the most infections ones are self-limiting, lasting a few days (Scherer et al., 2008). However, in some patients, the infection may be more serious and the dehydration can be life threatening. In these cases, as well as when Salmonella causes bloodstream infection, effective antimicrobials are essential for treatment. Salmonellosis has also been associated with long-term and sometimes chronic effects e.g. reactive arthritis (Colmegna et al., 2004).

There are numerous food-borne sources of Salmonella, including a wide range of domestic and wild animals and a variety of foodstuffs covering both food of animal and plant origin. Salmonella infection occurs when organisms are introduced in food preparation areas and allowed to multiply on food, e.g. inadequate storage temperatures, inadequate cooking and cross contamination of ready-to-eat food (Kureljusic et al., 2017).

The common reservoir of Salmonella is the intestinal tract of a wide range of domestic and wild animals, which may result in a variety of foodstuffs of both animal and plant origin (Obukhovska, 2013). Studies reported that dairy products (Richter et al., 2000), beef (Koochmaraie et al., 2012), fish (Onyango et al., 2009), pork (Meyer et al., 2010), poultry meat and eggs (Fearnley et al., 2011), fruits and

vegetables (*Gautam et al., 2014*) are vehicles for *Salmonella* transmission to humans.

The aim of this research was to establish the presence of *Salmonella* in swab samples from pig carcasses. Swab samples were collected from 100 carcasses of pigs. Sampling was done during ten weeks, every week the day was rotated, in order to cover all days of the week. During sampling, the swabs were taken from the carcasses after stunning, then after the completion of processing before chilling and 24 hours after the start of chilling.

Material and Methods

The isolation of *Salmonella* spp. was performed according to standard SRPS EN ISO 6579: 2008, Microbiology of food and feed - Horizontal method for the detection of *Salmonella* spp. The isolation of *Salmonella* spp. from ileum content was performed according to the same standard SRPS EN ISO 6579: 2008.

For biochemical confirmation API 20E kit (BioMérieux®, France) was used for *Enterobacteriaceae* and other Gram-negative bacteria.

For the identification of *Salmonella* polyvalent and monovalent sera were used produced by Institute of Public Health of Serbia "Dr Milan JovanovićBatut" and the Statens Serum Institute (Denmark), according to the Kauffman-White scheme or ISO/TR 6579-3: 2014.

Results and Discussion

The presence of *Salmonella* spp. after stunning was determined in 41% of the carcasses (100 carcasses were examined) while after processing, *Salmonella* was confirmed in 2% of the tested carcasses. Detection of *Salmonella* after stunning was within a range of 0 to 90%, during ten weeks of sampling. After processing, *Salmonella* was isolated only in two carcasses both originated from the third week of the study Table 1.

Table 1. The presence of *Salmonella* spp.

Week	Number of carcasses	Number of <i>Salmonella</i> after stunning		Number of <i>Salmonella</i> after processing		Number of <i>Salmonella</i> after chilling	
		Number	(%)	Number	(%)	Number	(%)
1.	10	8	80	0	0	0	0
2.	10	6	60	0	0	0	0
3.	10	9	90	2	20	0	0
4.	10	6	60	0	0	0	0
5.	10	4	40	0	0	0	0
6.	10	5	50	0	0	0	0
7.	10	1	10	0	0	0	0
8.	10	2	20	0	0	0	0
9.	10	0	0	0	0	0	0
10.	10	0	0	0	0	0	0
In total	100	41	41	2	2	0	0

Accurate detection of *Salmonella* spp. in food provides an opportunity to prevent the contaminated food from entering the food supply. In this study, *Salmonella* was isolated from 47 (23.5%) swab samples after stunning. In the investigation done by Karabasil *et al.* (2012), 46.7% of examined pig carcasses were positive for the presence of *Salmonella* after stunning as well. These results suggest the possibility that many pigs had become contaminated during the slaughter process by cross contamination which is especially noticeable in bad hygienic conditions in slaughterhouse lairages. A significantly lower number of carcasses detected to be positive for *Salmonella* after processing, indicate the importance of using good hygiene and manufacturing practices in slaughterhouses. The occurrence of *Salmonella* in pig carcasses after processing is different from country to country. The reported percentage of the occurrence was 6% in Italy (Bonardi *et al.*, 2003), 0.2% in Switzerland (Sauli *et al.*, 2003), 5.3% in Great Britain (Davids *et al.*, 2000) and 4.7% in Germany (Käshbohrerat *al.*, 2000) during the period from 2000 to 2003.

Conclusion

Salmonellosis remains the second most common zoonosis in humans in the EU, despite a significant decreasing EU/EEA trend in confirmed salmonellosis cases since 2008. In the last 5 years (2013–2017), however, the trend has stabilised. In 2017, the number of reported confirmed human cases and the EU notification rate slightly decreased after a 3-year increase. Almost half of the MS reported a

decreasing trend during 2008–2017, but in the majority of those countries the trend has stabilised and the number of MS reporting a significantly increasing trend doubled in 2013–2017. This could be partly attributable to more complete reporting and improvements in the surveillance of salmonellosis in a few countries (EFSA 2018)

Preživljavanje salmonella na trupovima svinja u klanicama

Jasna M. Kureljušić, Alesandra Tasić, Jadranka Žutić, Branislav Kureljušić, Ljiljana Spalević, Suzana Vidaković, Dragana Ljubojević

Rezime

Salmonela je široko priznata kao najznačajniji zoonotski patogen sa ekonomskim i zdravstvenim uticajem na životinje i ljude. To je jedan od glavnih bakterijskih uzroka gastroenteritisa širom sveta. Organizam se takođe može preneti direktnim kontaktom sa zaraženim životinjama ili fekalnom kontaminacijom poreklom od zaraženih životinja i ljudi. Najčešći simptom ne-tifoidne infekcije salmonelom je akutni gastroenteritis. Ciljevi ovog eksperimenta bili su da se utvrdi status salmonele na trupovima svinja u klanici. Za detekciju salmonele, uzorci su analizirani prema ISO 6579: 2008. Prisustvo *Salmonella* spp. Nakon omamljivanja utvrđeno je u 41% trupova (ispitano je ukupno 100 trupova), dok je nakon obrade *Salmonela* potvrđena u 2% testiranih trupova. Detekcija salmonele nakon omamljivanja bila je u rasponu od 0 do 90%, tokom deset nedelja uzimanja uzoraka. Nakon obrade, *Salmonella* je izolovana samo u dva trupa, iz treće nedelje studije.

Ključnereči: *Salmonella* spp., trupovi svinja, klanice

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SEROPREVALENCE TO *MYCOPLASMA HYOPNEUMONIAE* IN GILTS AND SOWS

Jadranka Žutić¹, Olivera Valčić², Branislav Kureljušić¹, Dobrila Jakić-Dimić¹, Jasna Kureljušić¹, Nemanja Jezdimirović¹, Nemanja Zdravković¹

¹ Institute of Veterinary Medicine of Serbia, Belgrade

² Faculty of Veterinary Medicine, University of Belgrade, Serbia

Corresponding author: Jadranka Žutić, jadranka.zutic@gmail.com

Original scientific paper

Abstract: Respiratory diseases represent a significant problem in pig farming as they can cause significant economic losses all over the World. One of the most common causes of infection is *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) either as the primary cause of enzootic pneumonia or as one of the causative agents of Porcine Respiratory Disease Complex (PRCD). In our study we presented the presence of antibodies against *M. hyopneumoniae* in 94 blood sera of gilts and 84 blood sera of sows, originated from 4 commercial pig farms. For this purpose, the indirect ELISA test was used. Seroconversion was established in 11 (11.70%) sera of gilts and in 15 (17.85%) sera of sows. The percentage of positive sera differed among the farms, ranging in gilts from 0- 28.57%, and in sows 9.09-36.84%. The results of the study have shown that infection with *M. hyopneumoniae* is present in gilts on three and in sows on all four examined farms. Successful control of *M. hyopneumoniae* depends on the efficient prevention of the transfer of the infective agent. Good results can be achieved using strict implementation of reliable serological methods. The serological control of gilts prior to fertilization is of extreme importance, as it can prevent spreading of the disease within the herd.

Key words: *Mycoplasma hyopneumoniae*, antibodies, blood sera, gilt, sow.

Introduction

Respiratory diseases are a significant health problem in the pig industry and an endless challenge not only to veterinarians, but to all other structures which are involved in livestock production. They are present on all meridians, causing

substantial economic losses due to great fatalities, slow growth, poor feed conversion rate and costs for medical treatment (Baker, 2005; Bochev, 2007; Nathues et al., 2013).

Respiratory diseases are the result of a combined effect of multiple factors, out of which living conditions and infectious agents stand out (Hansen et al., 2010). Constantly striving for greater profitability, agglomerations counting a large number of animals on a restricted area have resulted in overcrowding. In such conditions, there is an overproduction of gases, dust, heat and bioaerosol particles which influence the air composition, and the organism of the animals (Đorđević et al., 2007; Maes et al., 2018). Besides poor keeping conditions, sudden temperature changes and the immunosuppressive effects of the microbial agents also add to the pathogenesis of respiratory tract diseases (Thacker, 2004; Shen et al., 2017; Maes et al., 2018).

By combining the effects of a number of factors, among which the most important are the breeding conditions and infectious agents, a disease known as Porcine Respiratory Disease Complex (PRDC) can develop (Fano et al., 2005; Bruguera i sar., 2006; Cheong et al., 2017). The most common relevant infective agents are viruses and bacteria. Among them *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*) is the most common pathogen. *M. hyopneumoniae* is the primary cause of enzootic pneumonia (EP) and a concurrent agent causing PRDC (Thacker, 2004; Nathues et al., 2013; Garza et al., 2018). Enzootic (mycoplasmatic) pneumonia is a chronic, clinically mild, infectious pneumonia with a long incubation period and a tendency to become endemic in a herd. It causes persistent coughing, stunted growth, sporadic „flare-up“ episodes of respiratory distress and a high incidence of pulmonary lesions in fattening pigs (Ameri-Mahabadi et al., 2005; Wilson et al., 2013; Li et al., 2019).

M. hyopneumoniae binds itself to the epithelial cilia in the proximal parts of the respiratory system, resulting in subsequent clumping and loss of cilia. These losses of ciliary structures result in a poor efficacy of the mucociliary apparatus, thus „opening the gate“ to secondary bacterial microbiota (Thacker, 2004; Maes et al., 2018). The agent is transmitted mainly in a vertical fashion from the sow to litter, and thereon horizontally from litter to litter (Fano et al., 2005; Nathues et al., 2013).

For years *M. hyopneumoniae* has been the cause of significant health problems in countries with intensive pig farming, including the Republic of Serbia. For these reasons we have set the aim to test the blood sera of gilts and sows for the presence of *M. hyopneumoniae* antibodies. Further on, according to the obtained results, to evaluate the present status of *M. hyopneumoniae* infections, as well as to compare the obtained results with the findings from previous studies. In order to

achieve the set goals we have opted for iELISA as a sensitive and specific serological test.

Material and Methods

Blood was sampled from 94 gilts and 84 sows on four pig farms. The “farrow to finish” farms were located on the Northwest of Serbia and had a capacity of 1000 sows each. On the farms the pigs were not vaccinated against *M. hyopneumoniae*. Blood samples were taken from clinically healthy animals. Blood serum samples were tested by indirect ELISA (HerdChek M.hyo: Mycoplasma hyopneumoniae, antibody test Kit, IDEXX).

Results and Discussion

Out of the total number of 178 tested blood serum samples 94 were taken from gilts and 84 from sows. Results of the presence of antibodies against *M. hyopneumoniae* are shown in Table 1.

Table 1. Results of the presence of antibodies against *Mycoplasma hyopneumoniae* in gilts and sows blood sera

Farm	Category	Number of samples	No. positive (%)	No. negative (%)
1	gilts	26	3 (11.53)	23 (88.46)
	sows	21	3 (14.28)	18 (85.71)
2	gilts	23	2 (8.69)	21 (91.30)
	sows	22	3 (13.63)	19 (86.36)
3	gilts	21	6 (28.57)	15 (71.42)
	sows	19	7 (36.84)	12 (63.15)
4	gilts	24	0 (0.00)	24 (100)
	sows	22	2 (9.09)	20 (90.90)
Total gilts		94	11 (11.70)	83 (88.29)
Total sows		84	15 (17.85)	69 (82.14)
Total animals		178	26 (14.60)	152 (85.39)

Antibodies against *M. hyopneumoniae* were established in a total of 26 (14.60%) samples from gilts and sows. In gilts, antibodies were detected in 11 (11.70%) samples and in sows in 15 (17.85%) blood sera samples. The percentage of positive sera differed among the farms, ranging in gilts from 0- 28.57% and in sows 9.09- 36.84%. Similar results were recorded in Slovakia, where seropositive animals

were detected on all four tested farms (Prokeš *et al.*, 2012), in Spain on all 12 tested farms (Sibila *et al.*, 2004), while in Poland out of 23 tested pig farms, 21 resulted *M. hyopneumoniae* seropositive (Dors *et al.*, 2012). Serological tests carried out in Northwest Germany have revealed that 65% of sows originating from 67 herds tested positive for *M. hyopneumoniae* antibodies (Grosse-Beilage *et al.*, 2009). In Belgium the percentage of seropositive pigs in clinically infected herds increased from 8% in pigs at 9 weeks of age to 52% in pigs at 18 weeks (Vicca *et al.*, 2002). In West India 459 pigs sera were analyzed by means of iELISA. Antibodies to *M. hyopneumoniae* were found in 8.71% pigs. A higher percentage of animals was established in pigs younger than 12 months (62%), seroprevalence in pigs older than 2 years was 22% (Vogler *et al.*, 2017). Interesting is the first report of *M. hyopneumoniae* seroprevalence in farmed wild boars in China. They have detected 78 out of 882 (8.8%) samples as seropositive (Liang *et al.*, 2018).

According to our results, farm number 4 stands out, as out of the 24 tested samples from gilts not a single one resulted positive on testing for *M. hyopneumoniae* antibodies. Also, on this farm the lowest number of seropositive sows was recorded (9.09%). This can be explained by the fact that this farm is newly build with highly technological up to date solutions. Contributing to its success is the fact that biosecurity measures, as well as health management are of the highest standard. The highest number of seropositive gilts (28.57%) and sows (36.84%) was recorded on farm number 3. Contrary to farm number 4, this farm has old inadequate facilities, which are difficult to maintain adequately and perform correctly measures such as ventilation and disinfection.

In general, it can be concluded that on all four farms *M. hyopneumoniae* infected animals were detected. The number of infected gilts was lower compared to the number of infected sows. When comparing the obtained results with the results we previously reported it can be noted that the overall number of infected animals has decreased. Thus, in the previous years the number of infected gilts on farms was 17-65% (Žutić *et al.*, 2008) and sows 12-67% (Žutić *et al.*, 2011). This trend can be explained by the fact that gilts are serotested before being introduced into the herd, regardless if they are bred on the farm or are acquired from other sources.

Conclusion

The results of the investigations have shown that infection with *M. hyopneumoniae* is present in gilts on 3 and in sows on all 4 examined farms. The intensity of infection differed among the farms, and also among the gilts and sows. The best results were obtained from farm number 4 where only 9.09% sows were seropositive and not one gilt. In comparison of both categories it can be noted that the

total number of infected gilts (11.7%) is lower than the number of infected sows (17.85%)

The successful control of *M. hyopneumoniae* depends on the efficient prevention of the transfer of the infective agent, both between the farms and also between certain categories of animals on the same farm.

Serological diagnostics of infections caused by *M. hyopneumoniae* is an essential mean for the identification of atently infected herds, the elimination of the infection from the herd and control of animals in quarantine.

The serological control of gilts prior to fertilization is of great importance as it prevents vertical and horizontal spreading of the disease within the herd.

According to the experience of a number of authors, ELISA test can be considered as a highly sensitive and specific serological test in the diagnostics of *M. hyopneumoniae* infections which can be used as a monitoring tool in different categories of pigs.

Seroprevalencija *Mycoplasma hyopneumoniae* kod nazimica i krmača

Jadranka Žutić, Olivera Valčić, Branislav Kureljušić, Dobrila Jakić-Dimić, Jasna Kureljušić, Nemanja Jezdimirović, Nemanja Zdravković

Rezime

Respiratorne bolesti predstavljaju značajan problem svinjarske proizvodnje nanoseći velike ekonomske gubitke u mnogim zemljama širom sveta. Nastaju kao posledica kombinovanog dejstva brojnih faktora a kao najznačajniji se smatraju uslovi držanja i infektivni agensi. Među najučestalije infektivne agense ubraja se *Mycoplasma hyopneumoniae* (*M. hyopneumoniae*). *M. hyopneumoniae* je primarni uzročnik enzootske pneumonije (EP) i jedan od najznačajnijih učesnika u nastanku kompleksa respiratorne bolesti svinja-PRDC. Enzootska ili mikoplazmatska pneumonija je hronična, klinički blaga, infektivna pneumonija svinja sa dugim inkubacionim periodom i tendencijom da postane endemična u zapatu.

Cilj rada je bio istražiti prisustvo antitela protiv *M. hyopneumoniae* u krvnim serumima nazimica i krmača sa komercijalnih farmi u Republici Srbiji. U tu svrhu korišten je indirektni ELISA test. Ukupno su sa 4 farme ispitana 94 krvna seruma nazimica i 84 seruma krmača. Antitela na *M. hyopneumoniae* utvrđena su u 26 (14,60%) uzoraka u obe grupe životinja. Upoređujući obe kategorije životinja zapaža se da je niži procenat inficiranih ustanovljen kod nazimica (11,70%) nego

kod krmača (17,85%). Procenat pozitivnih seruma razlikovao se među farmama i kretao se kod nazimica od 0-28,57% a kod krmača od 9,09-36,84%. Na sve četiri farme otkrivene su životinje inficirane sa *M. hyopneumoniae* i na svim farmama je utvrđen manji procenat inficiranih nazimica u odnosu na krmače. Upoređujući rezultate ovih, sa rezultatima naših prethodnih istraživanja, uočava se značajan pad procenta inficiranih životinja u obe kategorije.

Ključne reči: *Mycoplasma hyopneumoniae*, enzootska pneumonija, antitela, iELISA.

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THE OCCURRENCE OF RESIDUE OF ANTIBIOTICS AND SULPHONAMIDES IN DIFFERENT TYPES OF HONEY

Dragana B. Ljubojević Pelić¹, Suzana Vidaković¹, Sandra Jakšić¹, Miloš Pelić¹, Jelena Vranešević¹, Jasna Kureljušić², Brankica Kartalović¹, Milica Živkov Baloš¹

¹Scientific Veterinary Institute "Novi Sad", Rumenački put 20, 21000 Novi Sad, Serbia

²Scientific Veterinary Institute of Serbia, Vojvode Toze 14, 11000 Belgrade, Serbia

Corresponding author: Dragana B. Ljubojević Pelić, dragana@niv.ns.ac.rs

Original scientific paper

Abstract: The presence of antibiotic residue in honey may cause serious threat for human health including allergic reactions, teratogenic, mutagenic and carcinogenic effects and development of antibiotic resistance. The aim of the present study was to determine residue of antibiotics in commercial samples of honey. One hundred and four commercial honey samples were collected randomly and examined for the presence of antibiotic and sulphonamides residue by using four plate method. The results showed that one sample (0.96%) was positive for antibiotics and that positive sample was detected from plate pH 6 seeded with *Bacillus subtilis* that indicate the presence of penicillin-type and tetracycline-type antibiotic residue. There is a need for continuous monitoring of honey in order to preserve its safety for consumers.

Key words: honey, food safety, residue, antibiotics

Introduction

Honey is known as a food that is associated with many beneficial effects on human health. From nutritional point of view, it is important to determine the basic quality parameters of honey. Moreover, from a health point of view, it is very important to assess honey safety. It should be emphasized that honey has an important role as an important bio-indicators of environmental pollution (Babić *et al.*, 2017). Potential environmental contaminants in honey include antibiotic residue, heavy metals, pesticides, radioactive isotopes, pathogenic microorganisms and organic pollutants. Improper beekeeping practice is the main reason for occurrence of antibiotic residue in honey. Antibiotic residues in honey originate mostly from the environment and inadequate beekeeping practices (Baggio *et al.*, 2009). According to Reybroeck *et al.* (2012) antimicrobials of interest in beekeeping are:

tetracyclines (oxytetracycline), aminoglycoside antibiotics (streptomycin), sulfonamides, macrolide antibiotics (tylosin, erythromycin), lincosamides (lincomycin), chloramphenicol, nitrofurans, nitroimidazoles, quinolones (fluoroquinolones), fumagillin as well as other antibiotics and chemotherapeutics. *Jakšić et al. (2018)* highlighted the importance of strict monitoring system and validation of analytical methods in order to achieve an accurate, precise and reliable data of acceptable minimal concentrations of antibiotic residues in honey. Risks related to the presence of antibiotic in honey are allergies, food processing problems and development of antibiotic resistant bacterial strains (*Capita and Alonso-Calleja, 2013; Strayer et al., 2014*). Consumer protection is of a crucial importance which is evident from the fact that the use of antibiotics in beekeeping, the maximum permissible levels for residue of antibiotics and regular monitoring are incorporated into the legislation of all developed countries (*Ljubojević et al., 2015*). Having in mind all the above mentioned facts, the objective of this study was to investigate the presence of antibiotic residues in honey samples collected during 2018 in Vojvodina, Serbia.

Material and Methods

A total number of 104 honey samples included 34 samples of acacia honey, 3 samples of floral honey, 15 samples of linden honey, 35 samples of meadow honey, 6 samples of honeydew honey, 3 samples of sunflower honey, 1 sample of rapeseed honey, 2 samples of bakery honey, 4 undefined samples and one sample of propolis were obtained from beekeepers in Vojvodina province. All samples were collected in their original packages and were transferred to the laboratory and kept at cold and dark place until analysis. Disc diffusion microbial inhibition test was performed using three media seeded with *Bacillus subtilis* at different pH values (6, 7.2 or 8) and one medium seeded with *Kocuria rhizophila*. The positive result was confirmed by HPLC. The HPLC analyses were performed with a Thermo Ultimate 3000 model by Thermo Fisher Scientific coupled with a photodiode array detector and Fluorescence Detector (FLD). The HPLC system was controlled by Chromeleon software. The method determination of oxytetracycline (OTC) was a modified method, prepared in accordance with ISO 17025. One hundred and four commercial honey samples was sampled and examined for antimicrobial drug residues. Standard solutions of antibiotics were used as control.

Results and Discussion

The results of this study showed that one sample (0.96%) was positive for antibacterial substances. The complete inhibition of growth on the surface of the agar around discs with an inhibition zone of 4 mm on one or more plates indicated positive results. One sample of linden honey was positive and it was detected on medium 1 (plate pH 6 seeded with *B. subtilis*) with inhibition zone of 10 mm that indicated the presence of tetracycline-type or penicillin-type antibiotic. The result was confirmed by HPLC and the OTC residue was detected. The results of our investigations on the antibiotic residues presence in honey samples on the territory of Vojvodina were published previously (Apić *et al.*, 2014, Apić *et al.*, 2015; Živkov Baloš *et al.*, 2017; Babić *et al.*, 2017). Apić *et al.* (2015) reported that the investigations of the presence of antibiotic residues in honey result in 2.59% of positive findings (5 out of 193 examined samples). Živkov Baloš *et al.* (2017) performed quantitative analysis of residues of tetracycline, streptomycin, chloramphenicol and sulphonamides in 100 samples of six different honey types using ELISA. According to their results all analysed honey samples were in compliance with the Regulations related the presence of tetracycline, streptomycin and sulphonamides. However, they observed that 12 out of 36 analysed samples of honey (30%) were positive to chloramphenicol and to sulphonamide residues. One sample (2,8%) was positive to both chloramphenicol and sulphonamides. Analyses were performed according to honey type and obtained results showed that sunflower honey was free from residues off all tested antibiotics, sulphonamides residue alone was found in forest honey, while the highest rate of chloramphenicol residues was determined in linden and mixed honey (83.3%). Dugalić-Vrندیć *et al.* (2005) tested 100 honey samples originated from Belgrade market places and supermarket by modified four plate method and reported the presence of antibiotic residues in 13 samples (7.8%) from market places and 5 samples (2%) in samples from supermarkets. Moreover, according to results obtained by Dugalić-Vrندیć *et al.* (2011) related to investigated residue of antibiotic in 65 samples of honey by two inhibitory methods (Premi® test and Four plate methods) the presence of residues was detected in 8 (12.31%) samples. Mujić *et al.* (2011) determined antibiotic residue in 46 samples of honey produced in Bosnia and Hercegovina by disc plate method and found no antibiotic residues. Saridaki-Papakonstadinou *et al.* (2006) reported tetracycline residues in 29% of examined honey in Greece. Ortelli *et al.* (2004) detected residue of chloramphenicol in 17% of honey samples in Switzerland and they also reported that the most of examined samples were imported from Asian countries. Gunes *et al.* (2008) found residues of erythromycin in 8% of honey samples in Turkey, while Vidal *et al.* (2009) found residues of erythromycin in 18.75% samples of honey in Spain. Reybroeck (2003) reported

that 1.61%, 2.77%, 4.16% of examined samples of honey produced in Belgium in the period 2000-2001 was positive for residue of streptomycin, tetracycline and sulphonamides, respectively while all examined samples were negative for the presence of β -lactam antibiotics and chloramphenicol. On the other hand, they found residue of streptomycin, tetracyclines, sulphonamides and chloramphenicol in 50%, 29.59%; 31.63% and 47.06%, of examined imported honey samples, respectively. Their results showed that a great part of honey imported from China and Asian countries contains antibiotic residue which is in accordance with many above mentioned studies. According to Commission Regulation (EU No. 37/2010 and amendments) honey should be free of antibiotic residues since no maximum residue levels (MRLs) have been proposed and honey containing residue of antibiotic cannot be sold in most of EU countries. However, some countries have set action limits, recommended target concentrations, non-conformity or tolerance levels for same classes of antibiotic in honey. In Great Britain the limits for tetracyclines and sulphonamides are 50 $\mu\text{g/kg}$, while in Belgium the limits for tetracyclines and sulphonamides have been fixed at 20 $\mu\text{g/kg}$, in France the limit for tetracycline in honey is 15 $\mu\text{g/kg}$ and in Switzerland 20 $\mu\text{g/kg}$, but since 2009 Switzerland stopped the use of tolerance levels (Reybroeck *et al.*, 2012). Also, In Republic of Serbia no MRL of antibiotic residues have been established (*Official Gazette SRJ*, no. 5/92, 11/92 and 32/2002 and *Official Gazzete RS*, no. 25/2010 – another regulation and 28/2011 – another regulation). Microbial inhibition assays, such as multi-plate tests are still widely used due to the fact that such tests are rapid, simple and cheap (Pikkemaat, 2009). Also, the simplicity and low cost of inhibitory tests make them suitable for analysis of the large number of samples in monitoring programs (Pyun *et al.*, 2008). The modified method 4 plates can be used as a first step in screening procedures. It can be used to identify the presence of antibiotic residues in samples of honey, but cannot be used for quantification of any antibiotic in honey. The final identification and quantification of antibiotic in honey request the use of an appropriate confirmative technique. Residues of antibiotic or their metabolites can be stable for a long period in honey due to the fact that the antibiotics are not actively metabolized by honeybees. Furthermore, elimination of the residue of antibiotics or their metabolites can occur only by consumption by the bees or removal by the beekeeper (Reybroeck *et al.*, 2012). Thus, relative high number of detection of residues of antibiotic in honey can be still expected. Prica *et al.* (2015) stated that the lack in quality of honey is mainly due to insufficient knowledge of beekeepers and lack of adequate equipment. They suggested that better compliance of honey quality with the relevant Regulation can be accomplished by regular practical training of beekeepers offering relevant knowledge and skills on the production process, storage, package and distribution

of honey. These recommendations could be also linked to the lack in safety of honey.

Conclusion

The presence of antibiotic residue in honey is mainly due to improper beekeeping practice. The present results showed that only one sample of linden honey (0.96%) was positive for the presence of antibiotic residue. The lack in safety of honey is mainly due to insufficient knowledge of beekeepers and lack of adequate equipment. Thus, honey safety could be improved by regular practical training of beekeepers offering relevant knowledge and skills.

Prisustvo rezidua antibiotika i sulfonamida u različitim vrstama meda

Dragana B. Ljubojević Pelić, Suzana Vidaković, Sandra Jakšić, Miloš Pelić, Jelena Vranešević, Jasna Kureljušić, Brankica Kartalović, Milica Živkov Baloš

Rezime

Prisustvo rezidua antibiotika u medu može prouzrokovati različite negativne efekte na zdravlje ljudi, kao što su pojave alergijskih reakcija, teratogeni, mutageni i kancerogeni efekti i razvoj rezistencije na antibiotike. Cilj ovog rada je da se utvrdi prisustvo antibiotika u komercijalnim uzorcima meda. Uzorkovano je sto i četiri uzorka meda i ispitano na prisustvo rezidua antibiotika i sulfonamida korišćenjem metode četiri ploče. Rezultati su pokazali da je jedan uzorak (0,96%) bio pozitivan na prisustvo antibiotika i to iz grupe penicilina ili tetraciklina pošto je pozitivan rezultat očitao na ploči sa pH 6 koja sadrži *Bacillus subtilis*. Postoji potreba za stalnom kontrolom uzoraka meda na prisustvo antibiotika na prisutvo antibiotika kako bi se obezbedila njegova bezbednost.

Ključne reči: med, bezbednost hrane, rezidue, antibiotici

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ECONOMICS OF SUSTAINABLE AGRICULTURAL PRODUCTION AND ANALYSIS MACROINVERTEBRATES OF WATER SOURCES IN SERBIA

Ivan Mičić¹, Zoran Rajić², Marija Mičić³

¹Faculty of Agriculture, University of Bijeljina, Bosnia and Herzegovina

²Faculty of Agriculture, University of Belgrade, Serbia

³Faculty of Technology, Leskovac, University of Niš, Serbia

Corresponding author: Ivan Mičić, divanlav@gmail.com

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Abstract: Within the framework of this research, the macroinvertebrate community has been explored in 14 water sources. These water sources differ in terms of temperature, diversity, anthropogenic impact, whether in open, semi-open or closed habitats. The yield is estimated from 0 without flow, 1: 1 <L / min, 2: 1 - 5 L / min, 3: 5 L / min, 4: 5 - 20 L / min, 5: 20 L / min. The temperature of the water varies from 10°C to 30.3°C, which is measured in the thermal spring. Based on the CCA analysis, it was established that temperature and yield, factors that largely affect the structure and composition of the macroinvertebrate community. The *Chironomidae* family is present in all explored water sources, except in Source 3, where the altitude is 1932 m and Source 12 is a thermal spring.

Key words: sources water, food, human and animal health

Introduction

The composition of the macroinvertebrate community in the sources is largely determined by abiotic factors such as: physically chemically (Orendt, 2000), altitude (Barquín and Death, 2006), type of substrate, hydrological (Ilmonen and Paasivirta, 2005), but also anthropogenic disturbance (Lindegaard et al., 1998), primarily by capturing sources (Dmitrović et al., 2016). In sources, the temperature is one of the most important factors affecting the composition and structure of the macroinvertebrate community (Bottazzi et al., 2011). Variation of yield has a major impact on the macroinvertebrate community, not only directly but also indirectly, as it also affects the composition and structure of the substrate (Von Fumetti, et al., 2012; Mori et al., 2006). For sources, there are constant environmental conditions, but there are variations in ecological factors at the source itself, eukrenon relative to the source stream, hypocrenon, and the sources are often habitats of relict species (Hayford and Herrmann, 1998; Di Sabatino et

al., 2003), or endemic species (*Boeters et al.*, 2013; *Radoman*, 1983). The boundary between eukrenone and hypocrenone is a difference in the temperature of 2°C (*Erman and Erman*, 1995), while according to *von Fummeti et al.* (2007) that is 1°C. *Pešić et al.* (2016) analyzing the difference in the composition and structure of the macroinvertebrate community between eukrenones and hypokrenones in Bosnia and Herzegovina, concluded that the temperature difference between these two sections is less than 1°C and that some taxa are characteristic for each zone. Sources in the area of Southeastern Serbia are slightly cultivated, but they have not yet been sufficiently explored, without collecting data on the biodiversity of macroinvertebrates of these habitats. A large number of habitats are endangered due to anthropogenic disturbance, primarily capture. Within this research, a community of macroinvertebrates was studied within 14 sources. These sources differ from each other according to the following characteristics: temperature, abundance, anthropogenic impact (whether captured or not), whether they are in open, semi-open or closed habitats. The goals of this research are:

- to determine the conditionality of the macroinvertebrate community with abiotic factors
- to determine the factors that are crucial for the formation of a macroinvertebrate community
- comparison of abiotic factors in eukrenone and hypocrene of the source
- comparison of macroinvertebrate communities of eukrenones and hypocrenons
- to determine the composition and structure of the macrozoobenthos community in different types of sources
- compatible macroinvertebrate communities in indoor and outdoor habitats
- to assess the impact of anthropogenic disturbance on the composition and structure of the macroinvertebrate community in the sources

All sources are located in Serbia, and the research covered the area within the following sub-areas: Koritnik, Dusnik, Soko Banja, Topilo, the Aleksinac area and the city area of the city of Nis.

Water sources near each other

Water from research sources is needed in the development of innovative technologies in all human activities, in the cultivation of high-yielding species, varieties, races and hybrids, in the selection for high productivity, the development of the so-called. Agrotechnopathy, population increase (*Pešić et al.*, 2016). Agricultural production is based on the exploitation of natural, mainly biological, chemical and physical resources. Soil is a physical source, i.e. Grain crop cultivation, field crops, fruit growing and viticulture and other plant species. In addition, it represents a chemical source that supplies crops with an adequate amount of macro and trace elements and other nutrients. It is also a source of nutrients needed for livestock production. Stable fertilizer is a chemical source of nutrients necessary for plant production, i.e. To maintain the quality of the soil and

soil, although the soil itself originates from biological sources, which is the result of livestock production. Plant and animal species represent biosources of agricultural production. Hydrosphere, and the Atmosphere, considered to be static, constituent parts of the Earth, are also physical sources. However, from the aspect of dynamics, They are very vulnerable, from the aspect of dynamics, degradable, exhausting chemical sources of plant and animal species. Particularly biologically, some of these sources can be restored, mostly extremely reproductive, while others are excellent, non-renewable or low reproductive. The goal of agricultural production is ultimately to increase the reproductive capacity of the above-mentioned sources. If agricultural production is organized and planned, the goal is achievable. According to EU estimates, by 2020, agricultural production is expected to meet food needs for the population. By 2020, the population will increase from 7 billion to 9.5 billion (*Haila, 1995*). Today, efforts are being made to stabilize consumption "within the boundaries" of the planet Earth. A stable concept of human development is precisely the fruit of conscience to keep in life everything that enables us to live. Agriculture resuscitations are included in this concept as part of ecosystems. For the stable development of all activities, Agenda 21, adopted in Brazil in 1992, has 40 chapters including 115 different programs, so Chapter 14 is "Sustainable Agriculture and Rural Development". The same chapter covers 12 different program ranges (45 to 56). Program no. 50, "The Role of Water in Stable Food Production and Stable Rural Development" deals with waters. Water has participated in all of the revolutions of humanity, it is a key factor (agrarian, cultural and industrial). Water as a natural resource represents an irreplaceable life cycle. Sources in the area of Southeastern Serbia have been slightly cultivated Wigger, et al., (2015), but have not yet been sufficiently explored, without collecting data on the biodiversity of macroinvertebrates of these habitats. A large number of habitats are endangered due to anthropogenic disturbance, primarily capture. In the explored area of the village of Mozgov, sources close to each other-Source in the forest were explored. The sources are small, about 2 m², without flow and without anthropogenic influence. In the same area, at different altitudes, there are 2 more sources, one without flow, the other with a small flow, small dimensions, without the influence of man. At the foot of the foothill, another source is analyzed without flow, and it is the only reoccurrent source, while the other four are lime-springs. More sources have been analyzed in the area of Soko Banja, one of which is thermal, reopsamokren, sealed and located in an open habitat, (grade 3). The other source in this area is the spring of the Moravica River, 10 m² in size, on the transient habitat, in the form of dams. In the vicinity, another source is analyzed, which is not captured, the estimate for the yield is 1, the smaller the dimension. The spring in the village of Resnik was analyzed, the source of larger dimensions, with a partially enclosed basin, and a generous, estimate-4; the source is located in a semi-open habitat. In the municipality of Niska Banja, in the area of Koritnik, 2 sources were analyzed, in the forest. One is a small dimension, captured by a rectangular type, with a flow1.

Also, the other is tapered in the form of a fountain, with a concrete trough, the yield is an estimate of 2. In the suburbs of Dusnik, two sources were analyzed, one was published, the estimate was 5. While the second source of the limestone type, without flow, was captured. One source was analyzed, in the area of Niš, which is captured in the shape of a fountain, grade 4, reoccluded, is in the open habitat. One source was analyzed, in the area of Topila, captured in the form of a fennel, repsocamperous type, grade 3, in the semi-open habitat (Table 1).

Table 1. Characteristics of investigated water sources

Date	Source	Water source	Name of the site	N	E	m. a. s. l. (m)
28. 08. 2018	1	Screwed, fountain	Sv. Bogorodica, D. Vrežina L1	44°20'21"	21°58'8"	189
			Sv. Bogorodica, D. Vrežina L2			
08. 08. 2018	2	Captured	Resnik, Vrelo, L1	44°38'56"	22°47'56"	415.9
			Resnik, Vrelo, L2			
08. 08. 2018	3	Captured	Ivor, Vrelo, 1, arch 1	42°59'838	22°88'2837	1932
	4		Ivor, Vrelo, 2, arch 1			
28. 08. 2018	5	With stone trough	Fountain Koritnik L1	44° 17'42"	23°0'21"	619
			Fountain Koritnik L2			
18. 08. 2018	6	Captured	Great source, Dusnik L1	44°09'15"	23°8'1"	489,9
			Great source, Dusnik L2			
18. 08. 2018	7	Without Any restoration	Source, Dusnik 2	42°09'39"	23°7'39"	540.2
18. 08. 2018	8	Without any restoration	Brška river, Source L1	44°17'49"	23°0'29"	529
			Brška River, Source L2			
08. 08. 2018	9	Without any restoration	The source of Brain, in the woods	44° 41' 40"	22° 41' 42"	378.5
08. 08. 2018	10	Without any restoration	Source Brain, with coins			
18. 08. 2018	11	Without any restoration	Brška river, a coarse source of stone	44° 17' 55"	23° 0' 29"	577.6
08. 08. 2018	12	Captured, fountain	Terme Soko Banja	44°39'52"	22°53'25"	287.2
28. 08. 2018	13	Captured	Niska spa vodica	44°18'29"	23°11' 49"	689
18. 08. 2018	14	Captured, fountain	Source Solvent	42°28'15	21°56'33"	629

*m. a. s. l. - metres above sea level

Source: Mičić, unpublished data

Materials and Methods

According to *Dmitrović et al. (2016)* and *Glazier, (2012)* measured at each location, 6 variables of oxygen concentration, oxygen saturation, temperature, pH, conductivity, yield was estimated according to the samples made using a handle of 10 to 10 cm diameter, with a diameter of 250 µm. Three locations are taken from the site, taking into account the taking of a subspecies with various substrates, shadows, vegetation. The network is upstream. Substance disturbance was performed prior to sampling, when the water was clear, the substrate was upstream with the mesh. Although it is not yet a universal operational definition of a sustainable system, sustainable development, or sustainable agriculture, it can simply be said that today's sustainable agriculture is "ecological". Agriculture itself is part of the ecosystem or micro agro-ecosystem. As such a subsystem, it undergoes all the changes taking place on a global scale. The consequence of permanent pollution of the ecosystem is only one of many causes of disappearance or complete extinction of some species of plant and animal species. As soon as one species disappears, a food chain in the biosphere is established, the survival of other species is also threatened, both in rising and in the direction of lowering the hierarchy, which is nevertheless cyclical. All this is the reason for preserving biodiversity. In order to better understand the significance of biodiversity for sustainable and stable agricultural production programs, it is necessary to define the definition of sustainable systems, agriculture in general, sustainable agriculture, as well as parameters or sustainability indicators, which was the goal of our research. By 2050, the population will rapidly grow from 7 billion to around 9.5 billion. However, in an effort to produce more and more food, there is an increasing risk of exhausting sources.

Results

The strategic plan of success in agricultural production is the protection of the environment and biodiversity. Since the goal of agriculture is to produce sufficient quantities of quality food for the human population, food production must be organized in such a way that it does not affect the quality of air, soil, surface and groundwater, and not disturb the existing balance in the biosphere, or the natural diversity of plant and animal genotypes, and phenotypes and their natural heritability. Agricultural production is said to be sustainable only if it is organized to ensure the viability of the biosphere at the same time, in conditions of increasing population growth (*Ilmonen and Paasivirta, 2005*). Biodiversity means the total number of existing plant and animal genotypes and phenotypes, i.e. Natural heritability, which maintains the variability of the genome of plant and

animal species. Within existing ecosystems, biodiversity maintains their permanent settings by enabling them to function, or to survive, as well as communicate with surrounding ecosystems. Agro-ecosystems, in the same way, i.e. Agricultural ecosystems affect their environment and vice versa. In preserving biodiversity means production that does not affect the balance in the biosphere, from the standpoint of agriculture, i.e. enables the survival of plant and animal genetic resources (bioresources) and contributes to their adaptability and future use in food production. Highly productive breeds in the production of animals, varieties and soybeans in the production of field crops, fruit growing and vineyards and investments are designed to preserve traditional biotopes and rare local breeds, species and varieties. In the agroecosystem, only a small part of the total biodiversity is crucial for the survival of the human population. Biodiversity in the context of agriculture must enable continuous food production for people living in different environments, but do not affect the evolutionary flow, i.e. Agricultural production must not cause a decrease in the number of existing natural varieties within the genome of plant and animal species belonging to other mini ecosystems in which a particular agroecosystem connects the (Haila, 1995). Production must be organized in such a way as to preserve and protect all existing genetic varieties within the agricultural ecosystem, in particular favoring the survival of plant and animal species, breeds, species and varieties adapted to all conditions of breeding, resistant to diseases specific to certain sites, which are not subject to agrotechopathies, ie. to those who are productive and who bring in different eco-geographical locations. Since genetic variability is conditioned by the intensity of selection, heritability, population size and breeding program, this means that the sustainability of biodiversity in the agroecosystem depends on these factors. The size of existing populations of these factors, plant and animal species is the most important for preserving biodiversity. Populations inevitably lead to a homozygous status that contributes to the reduction of genetic variability. Highly productive plant and animal species, all varieties, strains, races and even species that are low productive but resistant to disease and sustainable in all living conditions can disappear from agroecosystems. Within the agroecological system, it would mean agriculture as an unsustainable activity and would soon endanger the preservation of natural genetic variability and heritability in all other ecosystems with which they interact. We can simply say that the goal of agriculture is to close the food chain in which a person is the ultimate link. Short-term measures of selection, methods of breeding and cultivation of useful species, agricultural production can influence the reduction of genetic variability, the extinction of certain genetic or biological varieties. Extinction of them always leads to the loss of one link in the food chain, the chain is broken, and the person suffers, the one who organized this activity (Von Fumetti et al., 2007).

Discussion

The Ministry of Agriculture and Forestry in many countries adopts long-term development programs to avoid the consequences, given the specific requirements on the flatland, hilly and mountain eco-geographical locations (*Hayford and Herrmann, 1998*). With the past, the extinction of some plant species further affects total extinction or reduction, i.e. an increase in the number of inhabitants, invertebrates and vertebrates that are useful for agriculture (worms, birds, insects, rodents, etc.). The use of grazing in such situations, as degraded agroecosystems, has remained without taking any cultural practices to improve the situation. *Pešić et al. (2016)* state that the genetically-variable population adjusts to a lot of new living conditions in comparison with the genetic population produced exclusively for one productive purpose, such as high productivity. Organized growth targets must take into account all genotypic and phenotypic traits of a particular species, race, variety, animality, morphological and functional characteristics such as reproducibility, disease resistance and susceptibility to stress. The key issue for sustainable agriculture and biodiversity concerns relates to the cost-effectiveness of growing rare, low-productive biological resources within certain agroecosystems. *Heinonen (1995)* states that the model of sustainable agriculture, in the form of index and defined sustainability indicators. *Zimmermann, (2001)* states that further development of the sustainability model is correlated with all aspects of agricultural sustainability and includes eight major indices, each of which contains several variables as follows: - human component - effects of natural energy resources effects of environmental factors of economy - biological sources of biological efficiency (plants and animals) - social aspects - physical factors (soil, quality, erosion, etc.). *Wigger et al. (2015)* states that the three main indicators of sustainable agriculture are environmental and social factors, as well as the economy. Preservation of biodiversity and its significance for agricultural sustainability is not difficult to estimate and express in terms of the value of money (*Radoman, 1983*).

Conclusion

Based on our research, we conclude that there are three basic challenges. According to the quality that must be fulfilled, public use water has been divided into:

- The waters of unconditional quality for drinking water
- Conditional quality water for drinking water and
- Water without drinking quality.

Water quality should be used as the highest drinking water, for breeding and preparing food in animal husbandry. Degraded qualitative water can become a source of hydro-epidemic both for humans and animals, as well as the source of acute and chronic poisoning, and can have teratogenic, carcinogenic and mutagenic potential. The quality of water is determined based on its potential use and is regulated by the standards and regulations of the WHO and national health ministries. However, other standards for water used in agriculture and technological waters are also needed. On the other hand, technological water used in food production must have the same quality as drinking water used by humans. The same situation should be water used for domestic animal water from the source. For sources, the mean conditions are valid, but the variation of ecological factors at the source itself, eukrenon versus the flow of the source, hypocrenon. The boundary between eukrenone and hypocrenone is a difference in the temperature of 2 ° C. Analyzing the difference in the composition and structure of the macroinvertebrate community between eukrenones and hypokrenones in Bosnia and Herzegovina, concluded that the temperature difference between these two sections is less than 1 ° C and that some taxa are characteristic for each zone.

Ekonomika održive poljoprivredne proizvodnje i analiza makrobeskičmenjaka u izvorima vode Srbije

Ivan Mičić, Zoran Rajić, Marija Mičić

Rezime

U okviru ovog istraživanja, zajednica makrobeskičmenjaka je istražena u 14 vodnih izvora. Ovi izvori vode se razlikuju u pogledu temperature, raznolikosti, antropogenog uticaja, bilo na otvorenim, poluotvorenim ili zatvorenim staništima. Prinos je procenjen od 0 bez protoka, 1: 1 <L / min, 2: 1 - 5 L / min, 3: 5 L / min, 4: 5 - 20 L / min, 5: 20 L / min. Temperatura vode varira od 10°C do 30,3°C, što se meri u termalnom izvoru. Na osnovu analize CCA, utvrđeno je da temperatura i prinos, faktori koji u velikoj meri utiču na strukturu i sastav zajednice makrobeskičmenjaka. Porodica *Chironomidae* je prisutna u svim istraživanim izvorima vode, osim u izvoru 3, gdje je nadmorska visina 1932 m, a Izvor 12 termalni izvor.

Ključne reči: izvori vode, hrana, zdravlje ljudi i životinja

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POSTER SECTION III

EFFICIENCY OF PROTEIN UTILIZATION BY GRAZING RUMINANTS AND POSSIBILITY FOR IMPROVEMENT

Bojan Stojanović, Goran Grubić, Nenad Đorđević, Aleksa Božičković, Aleksandar Simić, Vesna Davidović, Aleksandra Ivetić

University of Belgrade, Faculty of Agriculture, Nemanjina 6, 11080 Zemun, Serbia

Corresponding author: Bojan Stojanović, arcturas@agrif.bg.ac.rs

Review paper

Abstract: Pasture has a high ruminal crude protein (CP) degradability (>70%) that results in poor utilization of pasture protein (22 to 25%) which is far from the theoretical maximum efficiency of 40 to 45%. Increased ruminal ammonia absorption not only represent the N waste and an environmental problem but also may impair animal performance. A better utilization of protein from fresh herbage is possible by matching the supply of rumen degradable protein and carbohydrates. Replacing a part of the N-rich pasture by some other forage (corn silage, low N hay) or concentrates with a low protein content and a low ratio of degraded protein and degraded carbohydrates may decrease N losses and increase the efficiency of ration protein utilization. The optimal ratio of N to fermented organic matter in rumen is around 25 g/kg. Grazing a pasture with a high water soluble carbohydrates content may provide a higher energy supply at the rumen. Addition of fibrolytic enzymes in diets for pastured ruminants through the supplemented dry feeds could increase dry matter digestibility and improve the nitrogen use efficiency. The increase of tannin content in diets for grazing ruminants may reduce the ruminal protein degradability and increase nitrogen efficiency use. Optimal grassland management and supplement feeding of grazing ruminants may markedly increase the efficiency of utilization of protein from pasture.

Key words: cattle, sheep, pasture, nutrition, nitrogen, utilization

Introduction

Pasture especially in an intensively managed grazing system is generally higher in rumen degradable protein (RDP) than similar forages harvested as silage and hay. This results in poor utilization of pasture protein due to rapidly and extensively ruminal degradation of CP from pasture, where a large proportion of

forage protein is rapidly transformed into ammonia by rumen microbes with the excessive ammonia-N absorption across the rumen wall and increased urinary nitrogen losses (Stojanović *et al.*, 2010). Furthermore, pasture based diets promote extensive absorption of NH_3 nitrogen from the rumen as greater proportions of NH_3 nitrogen are in the nonionized form because of the higher ruminal pH associated with these diets (Bach *et al.*, 1999). Increased ruminal ammonia absorption not only represent the N waste and an environmental problem but also may impair animal performance (Stojanović *et al.*, 2006a). Negative effects on the animal of lower N use efficiency and increased ruminal ammonia resorption are associated with reducing energy availability due to the energy spent in the conversion of NH_3 into urea, lower dry matter intake (DMI), reproductive problems and environmental pollution, mainly through excretion of urinary N (Stojanović *et al.*, 2007). Dairy cows are characterized with the low conversion of dietary N into milk N, ranging between 13 and 31% in grazing systems and 40 to 45% under confinement systems with balanced rations.

Synchronization of the availability of N and carbohydrate in the rumen is an important condition for optimizing microbial protein synthesis. Microbial growth and the efficiency of NH_3 utilization are highly determined by the availability of carbohydrates in the rumen. Energy supplementation of diets based on fresh forage may result in reduced ruminal NH_3 -nitrogen concentrations, and improved animal performance (Stojanović *et al.*, 2014). On the other hand, supplements containing excessive amounts or inappropriate types of carbohydrates may negatively affect ruminal fermentation. Better utilization of pasture CP in ruminants may be possible by matching the supply of RDP and carbohydrates. A ratio between 19.3 and 25 g of N/kg of OM fermented optimizes the efficiency of ruminal ammonia utilization (van Vuuren *et al.*, 1990). Under grazing conditions, energy is the main limiting nutrient for lactating cows' productivity, while CP supply usually exceeds animal requirements (Stojanović *et al.*, 2016).

The objectives of this study were to consider the possibilities to improve protein use efficiency and production performances of grazing ruminants by optimization of pasture utilization and supplementary nutrition.

Characterization of protein in pasture

In plants, proteins appear mainly in the form of enzymes, and since the main enzymatic process is photosynthesis, the most abundant protein in all green leaves is ribulose-1,5-biphosphate carboxylase/oxygenase (Fijałkowska *et al.*, 2015). This protein is the first enzyme in the Calvin cycle in chloroplasts, soluble in borate-phosphate buffer, and makes up to 50% of soluble protein, whereby soluble protein accounts for about 60-70% of CP in fresh herbage. The free amino

acids (AA) content of forage plants vary considerably, and makes 4 to 32% of total protein-N.

The CP from lush pasture is highly soluble and rapidly and extensively degraded in the rumen. Plant composition changes with maturation where there is an increased ratio of neutral detergent insoluble protein (NDICP) in CP and thus, an increased value for insoluble protein. With increasing maturity, there is the decline in total protein content of pasture. A decline in crude protein content of spring-grown herbage is 1.43 g/kg DM with each day after April 10 (to the day 100, *Givens et al.*, 1989). According to *Dinić et al.* (2002) the reduction in CP content from the beginning of heading to full heading growth stage of Italian ryegrass was from 13.1 to 10.1% DM and of perennial ryegrass from 16.4 to 12.2% DM.

Nitrogen fertilization is an effective way to increase grass production, which results in higher DM yields at a fixed date after N application and in a reduction in growing days to reach a particular production stage. With increasing levels of N fertilization, the N content and protein N of grass increases, but the proportion of protein N in total N declined from 74 to 60% with increase in the proportion of nitrate and nitrite N (*van Vuuren*, 1993). The absolute decline in N with increasing maturity is sharper in high fertilized grass.

Radiation stimulates the photosynthesis and the increase in carbohydrates will cause a dilution of CP. Enhanced leaf growth will dilute herbage N and therefore the CP content will decrease at higher temperatures (*Spiertz*, 1982).

Stojanović et al. (2018) reported a tendency of slight decrease of the CP values during the spring grazing period (15.3, 13.7 and 14.6% DM, for the 1., 2. and 3. cut) in herbage obtained in conditions of simulated rotational grazing on permanent grassland. However, *Tomić et al.* (2002) did not find consistent trend for CP content in herbage from 1, 2. and 3. cut of *Dactylis glomerata* (8.8, 10.1 and 10.1% DM), *Phleum pratense* (9.3, 12.53 and 8.6% DM), *Festuca pratensis* (10.7, 10.7 and 11.0% DM), *Lolium multiflorum* (9.0, 9.7 and 9.0% DM) and *Festuca rubra* (10.6, 10.1 and 9.25% DM).

Improvement of protein utilization in pasture ruminants

The pasture has a high ruminal CP degradability (>70%), and therefore provides smaller amounts of RUP compared with cows on TMR diets (*Stojanović et al.*, 2015).

Crude protein levels in temperate grass species vary widely and may range from 7.0 to 15.0% DM and up to 30.0 % DM where pastures are heavily fertilized. The rate of CP rumen degradation from grazed herbage ranges from 8.6%/h for nonfertilized pastures to 10.3%/h for pastures fertilized with 400 kg N/ha/year,

wherein for example the rate of ruminal protein degradation of corn silage is 1.4%/h (*van Vuuren, 1987*). The stage of herbal maturity affects rate of ruminal protein degradation, wherein for 3 weeks after mowing it is 12.2%/h and after 6 weeks of regrowth is 9.9%/h. As grasses mature CP content decreases, the rate of protein degradation in the rumen is reduced, while increases the proportion of herbage protein entering the small intestine.

The efficiency of utilization of N in dairy cows fed fresh herbage is 22 to 25% which is far from the theoretical maximum efficiency of 40-45%. Production of grazing dairy cows not receiving supplementation is limited to 20-25 kg/day by the restricted intake of energy. The excessive ruminal degradation of protein from fresh forages causes the excessive loss of N, which cannot be completely used for microbial protein synthesis, mostly due to an insufficient amount of easily degradable carbohydrates (*Stojanović et al., 2006b*). The increase of resorption of NH_3 through rumen mucous membrane, leads to increased levels of urea in blood, milk and urine (*Stojanović et al. 2006a*).

Non-structural (water-soluble) carbohydrates in herbage are dominantly glucose, sucrose, fructose, fructosans and amylose (starch). Sugars are metabolic intermediates and usually occur at low concentrations, whereas the fructosans are main storage carbohydrates (*van Vuuren, 1993*). With increasing maturity the content of fructosans increased until week 5 after this peak fructosans were translocated or synthesized into structural carbohydrates.

A better utilization of protein from fresh herbage is possible by matching the supply of rumen degradable protein and carbohydrates. The optimal ratio of N to fermented OM in rumen is around 25 g/kg. Usually the ratio of ruminally degraded N to carbohydrates in fresh herbage exceeds 25 g/kg, especially in young herbage where a high proportion of N may be present in the form of non-protein nitrogen. The rate of CP degradation of fresh herbage is relatively high: 8.0 to 14.0 %/h, thus for optimum synchronization the supplement should have a rate of carbohydrate degradation similar to that of herbage CP. The beet pulp is characterized by a high rate of carbohydrate ruminal degradation while the rate of ruminal degradation of corn is lower, around 5.0 %/h (*Tamminga et al., 1990*).

When young leafy grass is the main feed, supplementing the diet of grazing dairy cows with a low-protein, high-energy feed, increases the efficiency of N utilization. Using corn silage indicates an improvement in N utilization of 25 -30%.

Table 1. Nitrogen balance of cows fed fresh herbage or a combination of herbage and corn silage (51/49 on DM basis), (*van Vuuren, 1993*)

Ration	N intake, g/day	N excretion, g/day			Efficiency, %
		Milk	Faeces	Urine	
Herbage	626	107	158	361	17
Herbage + corn silage	494	118	178	198	24

The use of concentrates with low CP and high nonstructural carbohydrate (NSC) contents improves nitrogen use efficiency (NUE). Reducing CP content in the diet for dairy cows from 18 to 16%, through ration formulation, made possible to maintain milk production, reduce N intake by 10 to 15% and N excretion by 13 to 20% (*Satter et al., 2002*). Cows consuming only pasture had a nitrogen use efficiency of around 21.0% while supplemented grazing cows with carbohydrate feeds improved their NUE by storing in the milk 24.6% of the ingested N (*Keim and Anrique, 2011*). This improvement was due to a decrease in CP content of the diet and to an increase of microbial protein synthesis, and the improvement of NUE as a response to carbohydrate supplementation occurs mainly when the pasture has a CP content higher than 20%. In order to avoid a negative effect on the OM digestibility and efficiency of microbial protein synthesis (g N/kg digested OM), it has been suggested that grain inclusion should not exceed 24% of total diet. *Casper et al. (1999)* observed a tendency towards a higher nitrogen use efficiency when supplementing grazing cows with barley and soybean meal or with corn and extruded soybean meal, and determined lower amounts of ruminal $\text{NH}_3\text{-N}$ (barley and soybean meal), which indicates a better utilization of N for the synthesis of microbial protein. Milk production was higher in diets supplemented with corn as a consequence of a higher dry matter intake (DMI). Supplementation of 8.7 kg of corn for the grazing dairy cows (orchardgrass - *Dactylis glomerata*) with pasture DMI of 19 kg/day, decreased pasture DMI – 15.8 kg/day while increased total DMI – 24.5 kg/day, increased milk yield (20.7 to 29.8 kg/day), milk protein yield (0.60 to 0.90 kg/day), with increased milk protein percentage (2.96 to 3.10%), (*Bargo et al., 2002*). Supplementing of grazing dairy cows (perennial ryegrass - *Lolium perenne*) with 1.8 or 3.6 kg/day of concentrate (corn/dry beet pulp), decreased pasture DMI (17.1 to 16.5 and 16.8 kg/day) while total DMI was increased (17.1 to 18.3 and 20.4 kg/day), increased milk yield (24.0 to 25.0 and 26.6 kg/day) as also milk protein yield (0.77 to 0.82 and 0.86 kg/day) with increased milk protein content (3.25 to 3.28 and 3.26%), (*Dillon et al., 1997*).

Steers (BW 494 kg) grazing autumn grass (11 kg DM/day) which was characterized with high CP content, supplemented with concentrate based on barley with higher ruminal degradation rate of insoluble fraction (18.9%/h) showed tendency toward to the larger values for liveweight and carcass gain (1200 and 756 g/day) compared with those supplemented with concentrate based on unmolassed beetpulp with lower degradation rate of insoluble fraction (9.4%/h), which had 1112 and 684 g/day of liveweight and carcass gain, respectively (*French et al., 2001*).

Grazing a pasture with a high water soluble carbohydrates (WSC) content may provide a higher energy supply at the rumen and a lower N intake. It is possible to improve the WSC/CP ratio by reducing N fertilization, increasing

grazing intervals, using selected cultivars with high sugar content, or adjusting the pasture intake according to the daily patterns of WSC and CP contents of the plant.

Water soluble carbohydrates concentration in grasses is higher at sunset than during the morning as a consequence of sugar accumulation (especially sucrose) due to photosynthesis (*Hristov and Jouany, 2005*). The CP content is higher during the morning than at sunset, because there is a negative relationship between WSC and CP content. *Trevaskys et al. (2004)* determined the effect of offering a daily strip of pasture to dairy cows in the morning or the afternoon, observing an increase in DMI, milk yield, milk N content and nitrogen use efficiency when the cows entered the pasture in the afternoon. Forage samples that were collected at sunset have a wider WSC/CP ratio than those collected at the morning.

In study of *Miller et al. (2001)* the late lactating dairy cows fed a perennial ryegrass pasture, selected for a high WSC content, or a standard cultivar, wherein authors observed a higher digestible DMI (+ 1.5 kg/day), greater milk yield (+2.7 kg/d) and nitrogen use efficiency (+ 7%), and a lower proportion of urinary N (10%) in cows fed the high WSC cultivar.

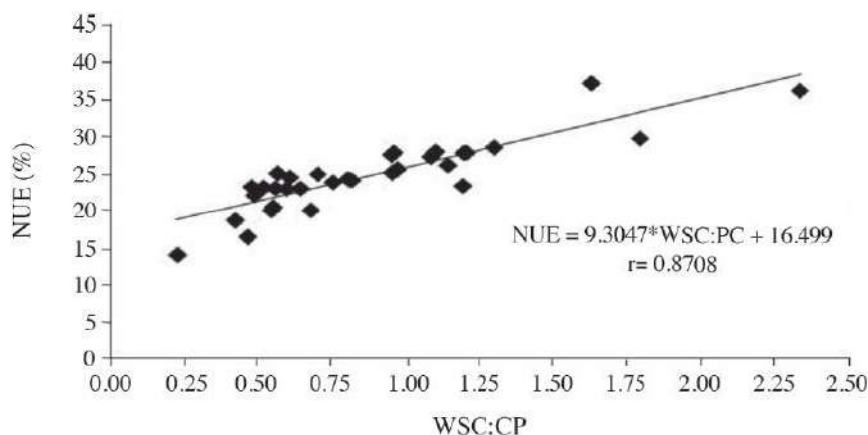


Figure 1. Nitrogen use efficiency (NUE) of dairy cows as affected by the water soluble carbohydrates (WSC) : crude protein (CP) ratio of the pasture (*Keim and Anrique, 2011*)

In study with sheep consuming either a high N perennial ryegrass silage (3.21% N in DM, supplied 19.5 g N/day) or a low N dried perennial ryegrass (1.83% N in DM, supplied 11.0 g N/day) *Siddons et al. (1985)* determined a higher efficiency of microbial N synthesis in the rumen with the low N dried grass, as also when the sheep were consuming high N grass silage they had a higher

concentration of ammonia in rumen fluid, a higher rate of irreversible loss of ammonia from the rumen and a higher rate of absorption of ammonia across the rumen wall.

The ruminal digestion of fibrous substrates, as the pasture is, may be improved by using fibrolytic enzyme supplements. In trial with cows in early lactation fed TMR, *Yang et al. (2000)* found that adding of fibrolytic enzymes (high xylanase and low cellulase activities) to concentrate increased the total tract digestibility of the dietary DM and CP (2.7 and 4.9%, respectively) as well as the milk yield (5.9%), while in the same experiment, the digestibility in lambs was not affected by enzyme supplementation.

Pasture with tannin containing legumes like sainfoin (*Onobrychis viciifolia*) or *Lotus pedunculatus* may reduce the ruminal protein degradability. Tannins have high affinity towards proteins, and by forming the complexes reduce soluble protein fractions amount. The tannin-protein bonds are cleaved under the acidic conditions in the abomasum, and protein can further be digested in abomasum and small intestine. Lactating cows fed with silage containing different levels of condensed tannins from birdsfoot trefoil (*Lotus corniculatus*) or with added Quebracho tannin extract at dosages of 15 and 30 g/kg DM had reduced milk urea nitrogen, ruminal ammonia and urinary N excretion (*Davidović et al., 2018*).

Conclusion

Efficiency of protein utilization from pasture is relatively low and consequently, a high proportion of ingested nitrogen is excreted in urine and faeces. Actual efficiency of nitrogen utilization in grazing cows is 15 to 25 %. Level of nitrogen fertilization, weather conditions, grazing intervals and plant maturity, selected cultivars affect the content and quality of grass protein. Supplementing the diet of grazing ruminants with low protein, high energy feeds increases the efficiency of nitrogen utilization, mainly due to an increase of microbial protein synthesis and reduce of nitrogen intake. Pasture with a high water soluble carbohydrates content provides a higher energy supply at the rumen, a lower N intake, improves microbial protein synthesis in rumen and nitrogen use efficiency. Using of fibrolytic enzyme supplements as also tannin containing plants can improve utilization of protein from fresh herbage. Grassland management and supplement feeding of grazing ruminants affect efficiency of use the protein from pasture, animal performance and the losses and excretion of N into the environment.

Efikasnost iskorišćavanja proteina kod preživara na paši i mogućnosti za unapređenje

Bojan Stojanović, Goran Grubić, Nenad Đorđević, Aleksa Božičković, Aleksandar Simić, Vesna Davidović, Aleksandra Ivetić

Rezime

Paša se odlikuje visokom ruminalnom razgradivošću SP (>70%) što za rezultat ima nizak nivo iskorišćavanja proteina iz paše (22 do 25%) što je znatno ispod teoretski maksimalne vrednosti od 40 do 45%. Povećana ruminalna apsorpcija amonijaka ne predstavlja samo gubitak u N i problem za životnu sredinu već utiče i na proizvodne performanse životinja. Bolje iskorišćavanje proteina iz sveže zelene mase moguće je postići obezbeđenjem optimalnog odnosa proteina i ugljenih hidrata razgradivih u rumenu. Zamenom dela paše koja je bogata u N, drugim kabastim hranivima (silaza kukuruza, seno sa niskim sadržajem N) ili koncentratom sa niskim sadržajem proteina i uskim odnosom razgradivih proteina i ugljenih hidrata u rumenu, mogu se smanjiti gubici u azotu i povećati efikasnost iskorišćavanja proteina iz obroka. Optimalan odnos N i fermentabilne organske materije u rumenu iznosi oko 25 g/kg. Korišćenje paše sa visokim sadržajem ugljenih hidrata rastvorljivih u vodi, obezbeđuje veću količinu iskoristive energije u rumenu. Dodavanje fibrolitičkih enzima u obroke za preživare na paši u dopunskom delu obroka, može povećati svarljivost suve materije obroka (uključujući SP) i poboljšati efikasnost iskorišćavanja azota. Povećanje sadržaja tanina u obrocima za preživare na paši, može smanjiti ruminalnu razgradivost proteina i povećati efikasnost iskorišćavanja azota. Optimalno korišćenje pašnjaka i dopunska ishrana preživara na paši mogu značajno povećati efikasnost iskorišćavanja proteina iz paše.

Ključne reči: goveda, ovce, paša, ishrana, azot, iskorišćavanje

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ALTERNATIVE SOURCES OF PROTEIN IN LAMB DIET

Dragana Ružić-Muslić, Milan P. Petrović, Zorica Bijelić, Violeta Caro Petrović, Nevena Maksimović, Bogdan Čekić, Ivan Čosić

Institute for Animal Husbandry, Belgrade-Zemun, 11080, Serbia

Corresponding author: Dragana Ružić-Muslić, muslic.ruzic@gmail.com

Review paper

Abstract: The high price of protein nutrients as well as import orientation have triggered the demand for alternative protein sources in the feeding of lambs. In addition, it is necessary to take into account the degree of degradability of proteins. In order to ensure optimal amino acid pool for high genetic capacity of lamb growth, it is important to provide a protein fraction that avoids degradation in the rumen. An excellent source of protein that slowly degrades in the rumen is fish meal. However, given the recommendation of its non-use, the effects of the use of Eko fish meal, as a fishmeal substitute in diet of lambs of average body weight of about 14 kg and age of 30 days, were examined. The source of protein did not significantly affect the growth rate and the efficiency of food utilization in the lambs of MIS population in fattening ($P>0.05$), suggesting that fish meal can be successfully replaced by Eco Fish Meal. As an alternative to soybeans, in diet for lambs in fattening, the following were used: sunflower meal with the addition of fibrolithic enzyme, lupine, fodder peas, fodder beans were used, while in the more exotic areas the following were used: castor cake, pomegranate peel, product obtained after rice fermentation without any harmful effect on the biological efficiency of growth and food conversion.

Key words: alternative sources of protein, lambs, soybean, fish meal

Introduction

Protein, in addition to energy, is a key determinant in the nutritional concept of lambs in fattening. The dominant source of protein in rations of younger ruminant categories is soybean. However, the high price, import orientation, fluctuations in production, global presence as genetically modified food, etc., impose the need for alternative protein sources (*Hadad, 2006; Hani et al., 2019*). Protein sources differ in terms of the amino acid profile as well as the degree of degradability (*Gleghorn et al., 2004; Bateman et al., 2005*). Dietary proteins reaching the small intestines of ruminants consist of two protein fractions:

microbial and protein non-degradable at the level of rumen. The microbial protein is created by the action of rumen microflora that breaks the dietary protein into peptides, amino acids and ammonia, after which these substances are used for the synthesis of their own proteins (*Ružić-Muslić, 2006*). During the degradation and synthesis there are some losses, (usually about 20%, but sometimes higher). A reduced amount of amino acids reaches the place of digestion and adoption of proteins, which means that the microbiological synthesis of proteins from the usual sources of protein and energy can not meet the needs of high-production meat breeds (*Ružić-Muslić et al., 2007, 2011-b*). Therefore, in order to provide the optimal amino acid pool for a particular production, it is necessary to provide a protein fraction that avoids degradation in the rumen (*Ružić-Muslić et al., 2007, 2011, 2014*). The correct selection of protein sources can affect the volume and rate of protein degradation in the rumen (*Grubić et al., 1992*).

Bearing all this in mind, the aim of this paper was to present results related to the effects of the use of alternative protein sources in nutrition of lambs in fattening.

The influence of protein source on the production performance of lambs in fattening

In search of alternative, environmentally friendly, sources of protein, *Alves et al. (2016)* have carried out studies on 40 male lambs, with average body weight of about 19.8 kg. Lambs were divided into 4 groups. As sources of protein in lamb rations, soybeans, castor cake, sunflower cake and sunflower seed were used. In analogue feeding treatments, the following average daily gains were achieved: 0.217; 0.190; 0.171; 0.135 kg. In spite of the higher content of lignin in the castor cake, which reduces the rate of degradation of the fibers in the rumen, and increases the retention time, which reduces the consumption of dry matter, compared to soybean, there was no significant difference in the realized gain in lambs in these treatments. However, in the treatment with sunflower cake and seed, as sources of protein, significantly lower growth compared to soybean was achieved, which resulted from lower digestibility of dry matter, related to higher content of ether extract and ADF.

By examining the effects of three sources of protein (soybean meal, sunflower meal, sunflower meal with added fibrolytic enzyme) on the performance of Shami kids of average body weight of 17.0 kg, *Titi (2003)* has determined following values for body weight: 30.23 kg, 29.68 kg, 36.38kg, daily gain: 0.155kg; 0.142 kg; 0.221 kg, respectively. The food conversion ratio was 6.61, 7.59, 4.70 kg/kg of gain, respectively. Therefore, the use of sunflower meal with the addition of fibrolytic enzymes has induced significantly ($P < 0.05$) higher

daily gain and more favourable food conversion ratio compared to the other two treatments. The superiority of this treatment is based on the fact that fibrolytic enzymes affect the reduction of fiber content and improve dry matter digestion, which results in providing enough energy for the growth of rumen microflora (Lewis *et al.*, 1995). The use of sunflower meal, as nutrient with best price on the market, is recommended in the diet of lambs, with the addition of fibrolytic enzymes, as a substitute for soybean.

Given that the production capacity of ruminants today is significantly improved, microbiological protein synthesis from common sources of protein and energy can not meet the needs of proteins in such animals, which means that the optimal ratio between proteins that degrade under the action of microflora of the rumen -degradable and proteins that avoid degradation in the rumen -non-degradable protein, must be provided (Grubić *et al.*, 1991).

Fishmeal is an excellent source of high quality protein that degrades slowly in the rumen (Can *et al.*, 2005) and has an excellent amino acid profile. Substitution of soybean, as a highly degradable protein source, with fish meal, improves the average daily gain and efficiency of food utilization (Orskov *et al.*, 1970, Beerman *et al.*, 1986). Orskov *et al.* (1970) have conducted research on lambs in fattening. As a protein source, fish meal was used in mixtures: 1, 6 and 12%. The achieved average daily gains in the above treatments are: 191, 270, 330g, respectively. Beerman *et al.* (1986), in their study of the effect of replacing soybean with 3% of fish meal, in diet for fattening lambs, have found improvement in average daily gain and food conversion ratio - 441 g and 3.52 kg, compared to - 350 g and 3.90 kg, in treatment without fish meal. In the research of Urbaniak (1994), the effects of different sources of protein were compared: blood meal, fish meal, soybean meal and casein, in isoprotein and isoenergetic diets for lambs of Polish Merino breed, of average body weight about 25 kg. In analogous treatments, average daily intake values were realized: 181, 197, 175 and 114 g. Results in concordance with the above mentioned are reported by Ponnampalam *et al.* (2005), emphasizing the effect of fish meal as a superior source of protein, compared to alfalfa hay, canola and soybean meal, on increased consumption of dry matter and average daily gain in lambs in fattening. The part of this answer can be attributed to improved fiber digestion in the rumen, which is the result of the availability of amino acids and ammonia for microflora action. Thus, the microbial protein is not sufficient to satisfy the metabolic requirements for amino acids, so the use of protein sources with high content of non-degradable protein, has resulted in superior performances.

However, according to Commission Decision 9/2001 on the protection of BCE (OJEC, 2001), food containing fish meal can not be produced in plants that produce food for ruminants, which in some ways leads to the distance to fish meal

as a high-quality protein source. In order to find alternative protein sources, research was conducted in order to examine the possibility of using Eco Fish Meal as a substitute for fish meal, in rations for fattening lambs (*Ružić-Muslić, 2018*). The trial included 40 lambs of MIS population, divided into 2 groups of 20 animals, age 30 days and average body weight of about 14 kg. The lambs in treatment I received as a source of protein fish meal, while the animals in treatment II received Eco Fish Meal replacement for fish meal. This product contains domestic nutrients of known origin such as genetically unmodified and thermally processed flour of ground soybeans, soy protein isolate, gluten, livestock yeast with the addition of minerals, amino acids, vitamins, enzymes and other supplements. Table 1 shows the production performance of the experimental lambs.

Table 1. Performances of trial lambs

Indicators	I	II	Level of significance
Initial body weight (kg)	14.55±2.61	14.57±2.93	NS
Final body weight (kg)	31.95±3.28	30.75±3.59	NS
Total gain (kg)	17.40±2.34	16.18±1.96	NS
Average daily gain, g	320±39.09	283±32.73	NS

I Fish meal (RB)

II Eco Fish Meal (EFM)

It is evident that the source of protein did not significantly influence the growth rate and efficiency of the food utilization in the lambs of the MIS population in fattening ($P>0.05$), suggesting that fish meal can be successfully replaced by Eco Fish Meal.

Karamnejada et al. (2019) carried out studies on 24 male lambs, with average body weight of 16.3kg. The nutritional treatment involved the use of protein sources in diet, which differed in terms of the level of degradable protein in the rumen. The lambs in the control group consumed a meal where the soybean flour was used as a protein component, while the animals in the treatment II received a meal containing 21% of the pomegranate peel and soybean, and the lambs in the treatment III - also 21% of the pomegranate peel and urea. diet II had lower, and diet II higher level of protein degradable in the rumen. Lambs on these treatments achieved the average daily gain of 0.221, 0.210, 0.182, and food conversion ratio 5.08, 5.83, 6.80 kg/kg of gain, respectively. The digestibility of

the dry matter was 689, 656, 610 g/kg, respectively. Lower daily gain and poorer food conversion ratio in treatments with pomegranate peel are probably the result of a negative effect on digestibility. The complete substitution of soybean flour with urea has adversely affected the growth rate and consumption of foods in lambs. It is generally known that the urea maintains a high level of protein that rapidly degrades in the rumen, which is not desirable for growing lambs. According to *Grubić et al. (1991)*, for each level of productivity, it is necessary that there is an optimal relation between the fractions of rapidly degrading proteins and those that avoid the degradation in the rumen.

Hani et al. (2019) have compared the effects of soybean and an alternative source of protein (*vinasse*), a product obtained after alcoholic fermentation of rice, on the performance of Texel lambs, of initial weight of about 30.48 kg, in a 60-day fattening. The nutritional treatment involved the substitution of the soybean protein with *vinasse* proteins: 250, 500 and 750 g/kg of DM, in rations for lambs. Animals in treatment with 500 g of this product have achieved a higher daily gain (0.140 kg) compared to the control group, as well as more favorable food conversion ratio, higher profit and economic efficiency. Increased daily gain is due to improved apparent digestibility of raw proteins and digestible energy, which implies the production of energy for fermentation in the rumen and increases the synthesis of the microbial protein. On the other hand, this treatment resulted in reduced production of methane, which is a consequence of the inhibitory action of *vinasse* on protozoa and methanogenic bacteria.

As an alternative source of protein, legumes are used in nutrition of lambs. Grains of leguminose have a high protein content, a significant concentration of energy and calcium. Their proteins are highly degradable in the rumen. They are most often used as an alternative to soybean flour (*Tudisco et al., 2010*). In addition, they are strategically important both in terms of reducing greenhouse gas emissions and the use of nitrogen fertilizers (*Carrouée et al., 2003*).

Lupine is a prospective leguminous crop. Lupine seed contains (300-500 g kg⁻¹) of crude protein and (50-100 g kg⁻¹) of oil, (*Calabrò et al., 2015*). In terms of fatty acid composition, lupine contains about 50-60% oleic acid, 16-23% of linoleic acid, and 8-9% linoleic acid (*Boschin et al., 2008; Calabrò et al., 2015*). It does not contain many antinutritive factors, found in soybeans (*Kung et al., 1991*).

Fodder peas is characterized by a lower content of crude proteins, compared with lupine (25-26% of dry matter), but with a higher level of lysine and methionine (*Saastamoinen et al., 2013*). At the same time, it contains 12.7% oleic acid, 35.3% linoleic and 5.4% linolenic acid (*Grela and Günter, 1995*).

Lestingi et al. (2016), have compared effects of lupine, peas, lupine and peas together, in diets for lambs of average weight of 16 kg and age of about 38 days, on production performances. Animals in the treatment I received 250 g of

lupine seed, in the treatment II: 150 g of lupine + 150 g peas, while lambs in the treatment III received 300 g of pea seed. In analogous treatments, the following average daily gain values were achieved: 0.140, 0.180, 0.210 kg, respectively. The food conversion ratio was 5.86, 5.16, 4.08 kg/kg of gain, respectively. In view of the observed properties, there were no statistically significant differences between the nutritional treatments, although the pea proved to be superior nutrient, given the achieved gain and more favorable food conversion ratio. The explanation for this phenomenon lies in the fact that lupine contains alkaloids, which adversely affect the consumption, the conversion of food, and thus the gain in lambs.

Lanza et al. (2003) have conducted comparative studies to compare the effects of soybean meal and pea (39 and 18% in the mixture), on the fattening performance and quality of the meat of Barbaresa lambs. The protein source has not significantly affected the average daily gain (0.218, 0.29, 0.250, respectively), food conversion ratio (4.7, 4.8, 4.1 kg), slaughter yield (50.1, 50.8, 51.2%), as well as physical and chemical properties of *M. longissimus dorssi*. Apparently, in all rations, the protein fraction was sufficient to meet the requirements of lambs in amino acids (*Ružić-Muslić et al., 2014*).

Morbidini et al. (2005) have examined the effect of soybean meal and beans on the performance of Italian Merino lambs. It was found that the use of beans in the meal resulted in a depressing effect on the performance of the lamb. The explanation lies in the greater content of NPN compounds and antinutritive factors. Therefore, the recommendations in the period after the early lamb weaning is: use in rations of proteins non-degradable at the level of rumen, since the rumen in this period is not fully functional and protein synthesis is less effective. In addition, for the same reasons, animals in this period are not able to neutralize possible anti-nutritional factors. The negative side of the leguminous grains is that a large part of them contains anti-nutritional factors: lecithin, trypsin inhibitors, tannins, saponins, phytase (*Ružić-Muslić et al., 2014*).

Conclusion

- Protein, in addition to energy, is a key determinant in the feeding concept of lambs in fattening.
- The dominant source of protein in rations for younger ruminant categories is soybeans.

- The high price, import orientation, production fluctuations, global presence as genetically modified food, imposes the need for alternative sources of protein.
- Fish meal is an extraordinary source of high-quality proteins, which are slowly degraded in the rumen, in the lamb diet.
- According to Commission Decision 9/2001 on the protection of BCE (OJEC, 2001), food containing fish meal can not be produced in plants producing food for ruminants, which in some ways leads to the distance to the fish meal.
- As alternative sources of protein, in the lamb fattening, the following can be used: Eco fish meal, sunflower meal with the addition of fibrolytic enzymes, lupine, peas, beans, without adverse effect on the biological efficiency of growth and food conversion.

Alternativni izvori proteina u ishrani jagnjadi

Dragana Ružić-Muslić, Milan P. Petrović, Zorica Bijelić, Violeta Caro-Petrović, Nevena Maksimović, Bogdan Cekić, Ivan Ćosić

Rezime

Visoka cena proteinskih hraniva kao i orijentacija na uvoz, inicirali su potražnju za alternativnim izvorima proteina, u ishrani jagnjadi. Pri tome, neophodno je voditi računa i o stepenu razgradivosti proteina. U cilju obezbeđenja optimalnog pula aminokiselina, za visoki genetski kapacitet rasta jagnjadi, veoma je važno obezbediti frakciju proteina koji izbegavaju razgradnju u rumenu. Odličan izvor proteina koji sporo degradiraju u buragu je riblje brašno. Međutim, obzirom na preporuku o njegovom nekorisćenju, ispitivani su efekti upotrebe korišćenja Eko fish meal (biljno visokoproteinsko hranivo), kao zamene za riblje brašno, u obrocima jagnjadi u tovu, prosečne telesne mase oko 14 kg i uzrasta 30 dana. Izvor proteina nije značajnije uticao na stepen rasta i efikasnost iskorišćavanja hrane kod jagnjadi Mis populacije u tovu ($P > 0.05$), što upućuje na zaključak da se riblje brašno uspešno može zameniti Eko fiš mealom. Kao alternativa soji, u obrocima

jagnjadi u tovu, korišćeni su: suncokretova sačma sa dodatkom fibrolitičkog enzima, lupina, grašak, pasulj, dok su na egzotičnijim područjima korišćeni: ricinusov kolač, kora od nara, proizvod dobijen nakon alkoholne fermentacije pirinča, bez štetnog uticaja na biološku efikasnost rasta i konverziju hrane.

Ključne reči: alternativni izvori proteina, jagnjad, soja, riblje brašno

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THE EFFECT OF CLIMATE CONDITIONS ON AFLATOXIN CONTAMINATION OF CEREAL GRAINS AND FEEDS

Vesna Krnjaja¹, Slavica Stanković², Ana Obradović², Tanja Petrović³, Violeta Mandić¹, Zorica Bijelić¹, Marko Jauković⁴

¹Institute for Animal Husbandry, Autoput 16, 11080, Belgrade-Zemun, Serbia

²Maize Research Institute “Zemun Polje“, Slobodana Bajića 1, 11185, Belgrade-Zemun, Serbia

³Institute of Food Technology and Biochemistry, Faculty of Agriculture, University of Belgrade, Nemanjina 6, 11080 Belgrade, Serbia

⁴Jugoinspekt Beograd ad, Čika Ljubina 8/V, 11000 Belgrade, Serbia

Corresponding author: Vesna Krnjaja, vesnakrnjaja.izs@gmail.com

Review paper

Abstract: Production and consumption of healthy food occur as the necessity of the modern life habits. The reduced risk of diseases improves the quality of life. Feed safety is a priority trend in all process of animal production. However, toxigenic fungal species and their secondary metabolites (mycotoxins) are the common feed contaminants. The aflatoxins and the producing *Aspergillus* species section *Flavi* in cereal grains and feeds has emerged as a serious concern with potential health hazards in humans and animals. This paper aimed to give an overview on of the effect of climatic factors on *Aspergillus* and aflatoxin contamination of cereals and feeds in agro-ecological conditions of Serbia and other European countries. Since the reduction of health risks and diseases in the livestock as consequences of aflatoxins presence in feed have become the priority tasks in feed safety control strategy thus the aim of this review has also been to recommend some of the measures for preventing their adverse effect.

Key words: cereal grains, feeds, *Aspergillus* spp., aflatoxins, climate factors

Introduction

The high-quality feed is a significant component of a successful animal rearing. Cereal grains (maize, wheat, barley, and ray) are the main components of feed for farm animals. However, infection of grains by toxigenic fungi is inevitable occurred in the field and during storage. Fungal development can reduce the nutritional quality of grains and provoke the production of their secondary

metabolites (mycotoxins) (Krnjaja *et al.*, 2012). Mycotoxins are toxic compounds and can cause health disorders in humans and animals (Zain, 2011). The one mycotoxin can be produced by several fungi, and that a fungus can produce several mycotoxins (Smith *et al.*, 2016).

The most common toxigenic fungal species belong to the genera *Alternaria*, *Aspergillus*, *Fusarium* and *Penicillium*. *Alternaria* and *Fusarium* are classified as field fungi, while *Aspergillus* and *Penicillium* species are considered as storage fungi, based on their different moisture requirements (Logrieco *et al.*, 2003). Field fungi require higher moisture conditions (20–21%) while the lower requirements of moisture (13–18%) is necessary for the development of storage fungi (Santin, 2005). The interactions of host crop, genotype, soil type, stress, and biological factors encourage the preharvest fungal development. The complex interactions of environmental factors (moisture, temperature, oxygen, and carbon dioxide), substrate, and biotic factors (insects and microorganisms) are favorable for postharvest fungal contamination (Miller 1995; Santin, 2005).

According to the reports of The Food and Agriculture Organization of the United Nations, approximately 25% of the cereals produced in the world are contaminated by mycotoxins (Reddy *et al.*, 2010). Mycotoxin contamination of cereal grains represents a potentially high risk for the health and production of farm animals (pigs, poultry and cattle). Mycotoxins are introduced into animal and human organisms most often through contaminated feeds and foods. Health disorders are most prominent in high-productive farm animals, given the significantly higher consumption of fodder. Factors such as the type and amount of mycotoxins in feed, duration of feeding with contaminated feed by the organism, species, breed, category, age, housing (rearing) of animals, etc. may also affect the degree of animal health disorders (Krnjaja *et al.*, 2009).

Aflatoxins, ochratoxins, fumonisins, zearalenone, deoxynivalenol, T-2 toxin, other trichothecenes, and ergot alkaloids are the most commonly detected mycotoxins in cereal grains (Binder, 2007). These mycotoxins cause significant economic losses in livestock production worldwide (Zain, 2011). Favorable environmental factors (high humidity and temperature) can increase the occurrence of mycotoxins in the crops. In particular, the occurrence of the aflatoxin is highly depended by the weather conditions. The global climate heating can cause that aflatoxins become a serious problem in grain production (Mannaa and Kim, 2017).

Considering that feed safety is a priority trend in all process of livestock production, the main aim of this review paper is to present the data on the effect of climatic factors on the natural occurrence of aflatoxins in cereal grains and feeds under agro-ecological conditions in Serbia (Europe). In addition, the aims have also been to indicate on potential hazards and harmful effects of these contaminants

in the animal production, and to recommend some of the preventive measures for the reduction of these contaminants in cereals and feeds.

Aflatoxins

Aflatoxins are difuranocoumarin derivatives produced by the *Aspergillus* species of section *Flavi* (Monge *et al.*, 2013). Four major aflatoxins founded in feedstuffs are aflatoxins B₁ (AFB₁), B₂ (AFB₂), G₁ (AFG₁), and G₂ (AFG₂), while two metabolic products, M₁ (AFM₁), and M₂ (AFM₂) has been detected as milk contaminants. *A. flavus* and *A. parasiticus* primarily produce AFB₁ and B₂, while *A. parasiticus* produce AFG₁ and G₂ (Ajani *et al.*, 2014). AFM₁ and M₂ are hydroxylated metabolites of AFB₁ and AFB₂, respectively, which are produced in the liver of humans and animals fed with contaminated foods/feeds and are secreted in the milk of mammals. Among the mentioned aflatoxins, AFB₁ is the highest toxic and most potent (Luongo *et al.*, 2014). According to the International Agency for Research on Cancer AFB₁ has been classified as the human carcinogen, Class 1 (IARC, 1993).

Aflatoxin occurrence in different types of feeds is a major risk for animal health, and causes high losses in livestock production. These toxins have been occurred all over the world in feedstuffs due to international trade in these commodities that contributes to their worldwide dispersal. They can also be found in animal-derived food namely meat, eggs, milk, and milk products if animals were fed with contaminated feed (Smith *et al.*, 2016).

AFB₁ was a first mycotoxin isolated from the feed and caused the death of 100.000 turkeys in 1960 in England (Bennet and Klich, 2003). Aflatoxins have been determined worldwide in different types of animal feeds, and their quantity varies depending on numerous factors. There are great differences in the levels of livestock feed contamination in particular years. In favourable years, such as warm and dry seasons, the fungi may accumulate aflatoxins before grain harvest. Also, as *Aspergillus* spp. tolerates low water activity, grain commodities under storage conditions can subsequently be contaminated (Bryden, 2012).

The poultry, pigs, and cattle are the most sensitive farm animals to aflatoxins, while sheep and rabbits are the least sensitive. Younger, gravid animals and males are more sensitive than mature ones, non-gravid, and females, respectively (Krnjaja *et al.*, 2009). Clinical signs and symptoms of aflatoxicosis in animals are vomiting, feed refusal, abdominal pain, pulmonary edema, gastrointestinal dysfunctions, fatty infiltration and necrosis of the liver, reproductivity disorders, anemia and jaundice (Talebi *et al.*, 2011; Ajani *et al.*, 2014). Aflatoxins may induce hepatotoxic, carcinogenic, teratogenic, and immunosuppressive effects on humans and animals (Smith *et al.*, 2016).

From that there are the risks by mycotoxin contamination, many countries have regulations for maximum permissible levels of mycotoxins in foods and feeds. According to Serbian regulation (*Službeni Glasnik RS 22/2018*), it adopted maximum permissible levels of total aflatoxins, aflatoxin B₁ (AFB₁) and aflatoxin M₁ (AFM₁) in different agricultural commodities and limits of AFB₁ in feedstuffs according to adequate regulations. These legislation limits are complied with EU regulations (*EC, 2010*), except for AFM₁. According to the mentioned Serbian and EU regulations, in all cereals and cereal products including also maize and rice, the maximum permissible level of AFB₁ and sum of aflatoxins (B₁, B₂, G₁, and G₂) have been set in 2–5 µg kg⁻¹ and 4–10 µg kg⁻¹, respectively.

According to the EU regulation (*EC, 2010*), the maximum level of AFM₁ is 0.05 µg kg⁻¹. However, in Serbia, the regulation limit of AFM₁ in milk is 0.25 µg kg⁻¹ (*Službeni Glasnik RS, 90/2018*). This limit has been reviewed every six months, since 2013 year, and has been determined based on available data from official laboratories on contamination of milk and feedingstuffs (*Jakšić et al., 2018*).

The European community set the maximum permissible level for AFB₁ to 20 µg kg⁻¹ for all feed materials (*EC, 2003*). According to the Serbian Regulation on the quality of animal feeds (*Službeni Glasnik RS, 27/2014*), the maximum level of AFB₁ for all feed materials is 30 µg kg⁻¹.

Natural occurrence of aflatoxins in cereal grains and feeds in European countries under climate change conditions

Contamination of cereal grains by *Aspergillus* spp. and higher occurrence of aflatoxins has been rare under climatic conditions of many European countries Austria, Belgium, Germany, Hungary, Poland, Romania, UK, etc. (*Lević et al., 2013*). In support of that, by mycotoxins analyzing of cereal grains samples collected during 2005–2009 in five countries from Southern Europe (Greece, Cyprus, Spain, Italy, and Portugal) *Griessler et al. (2010)* obtained the average aflatoxins levels which were not above EU regulation limits. However, in Northern Italy during 2003 it has been reported the intensive occurrence of *A. flavus* and aflatoxin presence in the maize crops (*Moretti et al., 2004*). The other one, in Northern Italy, it has noted the maize contamination by aflatoxins due to drought conditions in 2012–2013 favorable to *A. flavus* infection (*Perrone et al., 2014*). Likewise, aflatoxin contamination of maize was seven times higher in 2012 than in 2011 due to differences in weather conditions (*Accinelli et al., 2014*). Furthermore, in southeastern Romania, *Tabuc et al. (2009)* reported that 20% of maize samples collected during 2002–2004 had AFB₁ level above the adopted EU maximum limit (5 µg kg⁻¹) (*EC, 2010*). Similar to that, in Hungary, *Borbély et al. (2010)* have

determined 4.8% cereal and feed samples with AFB₁ levels above the EU limit. Then, in Serbia, the epidemic incidence of *A. flavus* and the increased aflatoxins levels in the maize grain crops during 2012 were determined. These intensive occurrences of *Aspergillus* spp. and aflatoxins can be explained with specific environmental and climatic conditions. Very high temperatures from the anthesis to maturity stages, then, the dry summers and the low seasonal rainfalls reported in 2012 in Serbia contributed to increase the development of *Aspergillus* spp. and aflatoxins levels in the maize crops (Kos *et al.*, 2012, 2014, 2017; Lević *et al.*, 2013; Krnjaja *et al.*, 2013; Obradović *et al.*, 2018). By analyzing maize kernel samples during 2012–2016, Kos *et al.* (2018) detected aflatoxins in 72.3%, 24.7%, 36.7%, and 5% of the maize samples in 2012 (range 1–111.2 µg kg⁻¹), 2013 (range 1.2–65.2 µg kg⁻¹), 2015 (range 1.1–76.2 µg kg⁻¹), and 2016 (range 1.3–6.9 µg kg⁻¹), respectively, while during the wet year of 2014, aflatoxins were not detected. Obradović *et al.* (2018) established AFB₁ level above a regulation limit in 16.7% of 90 investigated maize kernel samples in 2013 originating from production plots in Zemun Polje (suburb of Belgrade). However, Krnjaja *et al.* (2015, 2016, 2018) were not established the mean levels of aflatoxins above the regulation limits in post-harvest maize samples originating from different FAO maturity groups and different districts in Serbia in years 2013, 2014 and 2016. By analyzing the samples of different types of cereal grains (wheat, barley, oats, maize, and ray) after the harvest in 2012 from the main cereals growing area in Serbia, Kos *et al.* (2014) established aflatoxins only in the maize samples. Then, in Spain, the presence of aflatoxins under international regulation limits in stored barley grains collected from 2008–2010 has reported by Mateo *et al.* (2011). Similar that, in Poland, by analyzed mycotoxins in different genotypes of wheat, Rachoń *et al.* (2016) detected aflatoxins in trace amounts.

According to the results of Krnjaja *et al.* (2019), it has been reported that in 14.29% of chicken feed samples collected from different farms in Serbia during 2016, AFB₁ level was above the regulation limit. Similarly, in Republic of Srpska (Bosnia and Herzegovina), Dojčinović *et al.* (2017) have reported that AFB₁ levels were above the regulation limit (> 20 µg kg⁻¹) in one of 101, four of 85 and two of 86 tested samples of concentrated feed from 2014, 2015 and 2016, respectively.

Based on listed results, cereal grains and feed safety of fungal and aflatoxin contamination have been strongly influenced by climate conditions. The temperature, humidity, and precipitation are major climatic factors which directly affect fungal growth and infection. *Aspergillus* species have been occurred in tropical and subtropical regions in which droughts and high temperatures exist. Those factors also increase aflatoxin contamination of grain crops in the field. Until recently, *Aspergillus* species have not been often isolated in Europe, but in some years after the 2000s which are connecting with hot and dry summers the

occurrence of *A. flavus* and aflatoxin contamination of maize crops greatly increase (Van der Fels-Klerx *et al.*, 2016). *A. flavus* can cause pre- and post-harvest contamination of cereal grains. Although, the soil is the native habitat of this species, many types of organic substrates can be invaded by this fungus. Since aflatoxins are synthesized under the high moisture and temperature conditions, *A. flavus* produces them commonly in warmer, tropical and subtropical climates. So, in the European countries, the moderate climate should not be the risk of aflatoxins occurrence in cereals and other agricultural products. However, the effects of global climate heating started to influence, that aflatoxins become more prevalent in countries with the moderate climate (Baranyi *et al.*, 2013). Serbia with continental to moderate continental climate has also been under the risk of the effect of climate changes which may contribute to increased aflatoxins occurrence in cereals. In the future, it is predicted that AFB₁ will become inevitable in production of maize in Eastern Europe, the Balkan Peninsula, and the Mediterranean regions (Milicevic *et al.*, 2019).

Additionally, Battilani *et al.* (2016) reported the prediction of aflatoxin contamination in maize and wheat crops under a +2°C and +5°C climate change scenario where AFB₁ will become a feed safety issue in maize in Europe, especially in the +2°C scenario which will be expected scenario in the upcoming years.

Mitigation of aflatoxin cereal grains and feeds contamination

The control strategy of mycotoxin contamination in cereal grains and feeds consist of prevention of fungal development and mycotoxins production and detoxification of existing mycotoxins.

The preventive pre- and post-harvest measures, such as the healthy seeds, early sowing, harvest in full maturity, good storage conditions can be used to reduce fungal and aflatoxin contamination of cereal grains. Regular and correct ventilation and aeration of storage facilities may also prevents the increase in fungal contamination. Generally, only the dry grains with the moisture content of approx. 13% should be stored (Krnjaja *et al.*, 2009).

Physical adsorption, chemical, and biological detoxification methods are used for adsorption and degradation of toxic into non-toxic compounds. In practice, zeolite and clay minerals are used as adsorbents of aflatoxins. They are from a group of aluminosilicate minerals. Also, some of the biological adsorbents, such as modified glucomannan (isolated from cell walls of *Saccharomyces cerevisiae* yeast) are applied for inactivation of aflatoxins (Devegowda and Murthy, 2005). Individual or combinations of organic acid (propionic, sorbic, acetic acids, etc.), salts of organic acid (calcium propionate and potassium sorbate) and copper

sulphate are some of the chemicals which can inhibit fungal growth and proliferation (Akanke *et al.*, 2006). Lactic acid bacteria (LAB) are used in silage feeds. Probably, organic acid by LAB inhibits fungal growth, or there is the competition for nutrients in the substrate between fungus and LAB. Additionally, growing in the culture medium the bacteria can adapt faster than fungi. Then, binding AFB₁ and AFM₁ by LAB influenced by specified strains, matrix, temperature, pH, etc. The binding is a slightly reversible phenomenon. However, this biocontrol method could be a promising measure against fungal growth and aflatoxins in feeds (Ahlberg *et al.*, 2015).

Conclusion

It has been predicted that aflatoxins will continue to be of increasing importance in cereal grains and feeds. Therefore, there is a need for detailed information on the effect of climate conditions on the aflatoxin production by *Aspergillus* spp. Determination of climate factors will be the crucial task to control strategy and risk assessment of aflatoxin contamination. Monitoring of weather conditions in certain regions and constant control of aflatoxins level in cereals and feeds are necessary for health risk assessment of humans and animals.

For successful livestock production, it is necessary to ensure both healthy and high-quality fresh components that are included in the feed mixtures, and ready-made mixtures without contaminants that may cause adverse effects in the production chain. Given the importance of the aflatoxins as a hepatotoxic and carcinogenic agent that causes aflatoxicosis in animals, the best solution to control the formation of this mycotoxin is to prevent the growth of fungi on maize as the main component of animal feed and also on other susceptible commodities. Pre-harvest and post-harvest measures should be raised to cereal grains safety by aflatoxin contamination.

Uticaj klimatskih uslova na kontaminaciju žita i hrane za životinje aflatoksinima

Vesna Krnjaja, Slavica Stanković, Ana Obradović, Tanja Petrović, Violeta Mandić, Zorica Bijelić, Marko Jauković

Rezime

Proizvodnja i potrošnja zdrave hrane je potreba savremenog načina života. Bezbedna hrana za životinje je prioritet u svim procesima stočarske proizvodnje. Međutim, toksigene vrste gljiva i njihovi sekundarni metaboliti (mikotoksini) su najčešći kontaminanti hrane za životinje. Aflatoksini i produkujuće *Aspergillus* vrste iz sekcije *Flavi* u žitima i hrani za životinje pojavljuju se kao ozbiljan problem sa potencijalnim opasnostima po zdravlje ljudi i životinja. Cilj ovog preglednog rada je da se razmotri negativan uticaj klimatskih faktora na infekciju/kontaminaciju zrna žita vrstama roda *Aspergillus* i aflatoksinima, kao i hrane za životinje u agroekološkim uslovima Srbije i drugih evropskih zemalja. S obzirom da smanjenje rizika koji ugrožavaju zdravlje i smanjenje bolesti u stočarstvu, kao posledica pojave aflatoksina u hrani za životinje, postaje prioritetan zadatak u strategiji kontrole bezbednosti hrane za životinje, cilj ovoga rada bio je, takodje, da se preporuče neke od mera.

Ključne reči: žita, hrana za životinje, *Aspergillus* spp., aflatoksini, klimatski faktori

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SACCHAROMYCES CEREVISIAE IN FEED FOR RUMINANTS

Marija Pavlović, Aleksandra Tasić, Ksenija Nešić, Snežana Ivanović

Scientific Veterinary Institute Serbia, Vojvode Toze 14, 11000 Belgrade, Serbia

Corresponding author: Marija Pavlović, majaspavlovic@gmail.com

Review paper

Abstract: *Saccharomyces cerevisiae* is widely used in feed for ruminants, and can be added to feed as live strains or cell walls derivatives. Numerous beneficial effects are proven. *S. cerevisiae* exert effects on ruminal microbiota, by reducing the amount of oxygen, favoring anaerobiosis and growth of cellulolytic bacteria. Furthermore, it improve the lactate to propionate conversion, thus stabilize the ruminal pH. Supplementing of *S. cerevisiae* will decrease the ammonium content and increase the synthesis of microbial protein. Yeasts cells have the ability to adhere a certain bacteria capable of causing disease on its surface, and so removing a significant amount of harmful microorganisms. Mannan oligosaccharide and β -glucan may act as a high-affinity ligand and it is considered to have enhanced the neutrophils phagocytic activity. The general pattern with ruminants receiving fungal feed additives have shown to improve milk production and decrease the somatic cells count, which is especially important in the transition period of dairy cattle. It can be concluded that the use of *S. cerevisiae* as feed additive with probiotic activity is justified, and could be a solution for persisting acidosis problems.

Key words: feed, yeasts, *Saccharomyces cerevisiae*, rumen, milk production

Introduction

Yeasts are unicellular eukaryotic fungal microorganisms that are important source of products with probiotic activity. It can be included in feed as live strains or as cell walls derivatives. It is proven the beneficial effect of yeast from genera *Saccharomyces*, *Kluyveromyces*, *Hansenula*, *Pichia* and *Candida* and within these genera, species *S. boulardii*, *S. cerevisiae*, *K. fragilis*, *K. lactis*, *C. saitoana* and *C. pintolopesii* (Kumura et al. 2004; Campeanuet al., 2002; Bovill et al. 2001).

Among them, *Saccharomyces cerevisiae* is widely used in feed for ruminants. Probiotic derives from *S. cerevisiae* shows an immunostimulatory effect, and

improvement in the digestibility of diet compounds (Muñoz *et al.*, 2016). Those products have a high nutritive value, and are rich in enzymes, fatty acids, vitamin B complex, unknown growth factors and amino acids (more than 40% of total dry matter) (Sontakke, 2012).

Use of *S. cerevisiae* yeast as feed additive can improve the ruminant environment thus prevent rumen microbiota disorders. Supplementation of diet will reduce the amount of oxygen, favoring anaerobiosis and growth of cellulolytic bacteria (Doleza *et al.*, 2011), and will decrease the ammonium content and increase the synthesis of microbial protein (Hirstov *et al.*, 2005).

One of the purposes of use of *S. cerevisiae* is to decrease excretion of polluting outputs like nitrogen-based compounds and methane, as well as to lower the risk of ruminant digestive carriage of human pathogens. Regarding that, *S. cerevisiae* is considered generally recognized as safe (GRAS) by the Food and Drug Administration (FDA) and thus is appropriate for use in animal feeds.

Commercially available products vary in the strain of *S. cerevisiae* used, number and viability of cells present.

According to Suarez and Guevara (2018), the selection criteria to select strains with probiotic activity are:

- Tolerance to high acidity.
- Resistance to bile salts.
- Adhesion capacity to intestinal cells.
- Direct antagonistic effect on enterobacteria and other yeasts.
- Antisecretory effect against the toxins of pathogenic microorganisms.
- Trophic effect on the mucosa through the production of polyamines.
- Immunostimulatory effect.

Effects on ruminal microbiota and ruminal pH

Live yeasts or additives based on dried yeast influence the ruminal fermentation in the following parameters:

- Stimulate the growth and activity of total anaerobic microbiota;
- Stimulate the growth and activity of cellulolytic bacteria;
- Enhance the lactate to propionate conversion;
- Stabilize ruminal pH;
- Decrease ammonia concentration;
- Reduce methane production;
- Favor the production of volatile fatty acids.

S. cerevisiae increases the number of cellulolytic bacteria in rumen, thus exerting the improvement of fiber degradation. Simulative effect of yeasts on

cellulolytic bacteria is considered the consequence of yeasts ability to use oxygen, and lower its concentration in rumen environment. Dissolved oxygen can be detectable in the rumen in the amount that disturb anaerobic conditions. Yeasts cells consume the oxygen particles that are trapped in the solid fraction of the ruminal content, consequently reducing the redox potential for -20 mV. Therefore, live yeast that can consume oxygen, decrease the redox potential of rumen fluid and make the most favorable environment conditions for anaerobic bacteria, thus exert the promoting effects on rumen microflora. Different strains of *S. cerevisiae* can differ by oxygen scavenging property of yeasts.

Carro et al. (2015) and *Newbold (1996)* proven the reduction of oxygen between 46 and 89% with the supplementation of feed with live yeasts. Consequently, total microbial count in rumen may increase for 30% (*Newbold et al., 1998*), better use of feed, increased production of energy and microbial protein that ranges between 10 and 20% (*Carro et al., 2015*).

The most important consequence of these reactions is the stabilization of ruminal pH, thus preventing the ruminal acidosis. Consumption of readily fermentable carbohydrates in high rate results in accumulation of lactic acid and steep decrease of ruminal pH. In the acidic conditions microbial balance is disrupted, and number of lactate-producing bacteria growth e.g. *Streptococcus bovis*, and lactate-utilizing bacteria and protozoa falls.

The use of live yeast selectively stimulates the growth of bacteria populations consuming lactate (*Megasphaera elsdenii* and *Selenomonas ruminantium*) and serves as a competitor with lactate producers, for the consumption of sugar. Since lactic acid is the primary cause of acidosis in dairy cattle, reducing its concentration can have a significant effect on the pH. Furthermore, propionate to lactate conversion happens on the partial pressure of O₂ in rumen. When partial pressure of O₂ is low, lactate to propionate reaction is favored, and vice-versa. Yeasts in rumen decrease the partial pressure of O₂, and enhance the less acidic fermentation pathway. This is mechanism by which yeasts added in small amount can affect digestive processes, and supply growth factors such as organic acids, B-vitamins and amino acids to rumen microbiota.

Ruminal protozoa have stabilizing impact by delaying fermentation. It compete with amylolytic bacteria for starch that is fermented slower rate, with the main end-products volatile fatty acids rather than lactate. Protozoa consume lactate and prevent lactate accumulation.

Stabil ruminal pH is the main factor that decreases the incidence of acidosis and therefore digestive problems, lameness and high somatic cell counts associated with this cause (*Marrero, 2005*).

Ruminal microbiota has the ability to reduce carbon dioxide into methane, in reactions in which is produced hydrogen. Hydrogen is then transferred to H₂-

utilising microorganisms in the process called “interspecies hydrogen transfer”. Consequentially, methane is eructated by ruminants at a rate of 400 to 500 litres per day in adult cattle, which represents a loss of 8–12% of the energy available in the diet. Furthermore, the ecological aspect of that problem is based on the fact that methane represents a greenhouse gas that can contribute 18–20% of the global warming effect (Sontakke, 2012).

It is considered that yeast cultures reduce methane production in four ways:

1. Increasing butyrate or propionate production;
2. Reducing protozoa numbers;
3. Improving animal productivity;
4. Promoting acetogenesis.

Saccharomyces cerevisiae potentially stimulate acetogenic microbes (which produce acetate from CO₂ and H₂) in the rumen, consuming H₂ to form acetate and thus potentially reducing CH₄ production. Some aerobic viable yeasts or fungi (*Saccharomyces* or *Aspergillus spp.*) added in very small amounts have been reported to reduce methanogenesis in the rumen. However, results of studies with different yeast strains are not consistent and it is considered to be a strain dependent.

Literature data about reduction of ammonia level in rumen in the presence of yeast are numerous, which indicate to the changes in nitrogen metabolism affected by yeasts. The mode of action is explained as a competition of *S. cerevisiae* cells with protelytic bacteria for substrate and energy source, along with direct inhibitory effect of yeast's small peptides on targeted peptidases. The crucial is adequate balance between soluble nitrogen and carbohydrate supply in diet for ruminants, when *Saccharomyces cerevisiae* could enhance microbial growth and decrease nitrogen loss.

Effects on feed intake and digestion

Fungal feed additives based on *S. cerevisiae* increase feed intake rather than alter feed conversion efficiency. Factors that affect the feed intake are numerous: palatability, the rate of fiber digestion, the rate of digesta flow, protein status etc. Yeasts products have a pleasant odor and the ability of yeast to produce glutamic acid could benefit the taste of feedstuffs supplemented with yeast culture. Some studies have shown increased dry matter intake and milk production when yeast was fed during periods of heat stress, possibly reflecting the role in aiding appetite during time of stress (Harris *et al.*, 2017; Galvao *et al.* 2005; Lesmeister *et al.*, 2004). Different factors such as the strain of yeast, the nature of the diet or the physiological status of the animal (Chaucheyras-Durand *et al.*, 2008), dose and

feeding strategy (*Lesmeister et al., 2004; Magalhães et al., 2008*) influence dry matter intake.

Health promoting effects and effects on immune system

The most reported health effects of *S. cerevisiae* have been on either the reduction of diarrhea and improvement of fecal scores (*Galvao et al. 2005; Seymour et al., 1995; Magalhães et al., 2008*). The presence of live yeast in the digestive system of animals causes a phenomenon called competitive exclusion, in which certain bacteria capable of causing disease adhere to the surface of the yeast, and thus removing a significant amount of harmful microorganisms (*Sosa et al., 2010; Suarez and Guevara, 2018*). The complex stable microbiota in the gut helps the animal to resist infections. Above mentioned effects yeasts depend of the various factors, level of pathogenicity, the housing conditions, feeding regimes and environmental factors among others.

Mannan oligosaccharide a yeast cell wall component can act as a high-affinity ligand offering competitive binding site options for gram-negative bacteria, which possess mannose-specific type-1 fimbriae. The immediate benefits are associated with pathogen removal from the digestive system without attachment and colonization. This phenomenon may elicit significant antigenic responses, thus enhancing humoral immunity against specific pathogens through presentation of the attenuated antigens to immune cells. In addition, this process may suppress the pro-inflammatory immune response, which is detrimental to production performance.

Furthermore, β -glucan, found in the outer wall of yeast, whose function is to stimulate the natural defense system of the body, allowing when a threat to health animals occurs, this responds quickly and efficiently. Therefore healthier and productive herds are obtained, coinciding with the point made by *Delia et al. (2012)* and *Valeriano et al. (2017)*, which ensure that there is evidence that probiotics can stimulate specific and unspecific immune response.

Oligosaccharides present in yeast cells could have enhanced the neutrophils phagocytic activity. However, the mechanism by which β -glucans stimulated the immune system is not clarified. For immunomodulatory effect, β -glucans have to move from the lumen and interact with the immune cells. It has been suggested that cells of *S. cerevisiae* yeast cannot penetrate the intestinal endothelium barrier while only pure β -glucan have immunomodulatory effects (*Wójcik et al., 2014*). It is likely that the whole yeast cell might not stimulate the immune system, but either β -glucan fragments or other alternative mechanisms are involved (*Alugongo et al., 2017*).

Effects on milk production and somatic cells in milk

The general pattern with ruminants receiving fungal feed additives have shown to improve meat or milk production. In dairy cows, the transition period is the most critical phase to maintain the health and production status of herd. Proper nutrition and management during this period is crucial. It is recommended to use some feed additives which are a group of feed ingredients that can cause a desired animal response in a non-nutrient role such as rumen pH shift, growth, or metabolic modifier. *Dawson and Tricarico (2002)* analyzed the results gained from 22 studies with a natural feed additive containing metabolically active *S. cerevisiae* strain 1026 (Yea-Sacc®1026) involving more than 9039 lactating dairy animals. He found an average increase in milk production of 7.3% in yeast-supplemented animals. Responses to supplementation were variable and ranged from 2 to 30% increase in milk production. Improved live weight gain has been observed in some studies.

The concentration of somatic cells in the milk is directly related to the infection status of the udder. If an infection occurs in the udder, it recruits leucocytes (somatic cells) to the scene in order to rid of the bacteria causing problems. The somatic cell count in milk is directly related to its quality. High somatic cell count in the milk is not desirable. Microorganisms do not adapt well to fluctuations in the rumen pH. The bacteria dying in the rumen liberate endotoxins that cause inflammation of the hoof and udder. Since yeast stabilizes the ruminal pH, the somatic cell recruits decrease.

Conclusions

Saccharomyces cerevisiae as feed additive has a positive effect on feed intake and digestibility, milk production and production performances. Beneficial and stabilizing effect on ruminal microbiota, and furthermore on ruminal pH is present.

There is need to overcome some limitations in use of yeast cells, like variation in the yeast strain used, its viability (survival during pelleting, drying, under acidic gastric pH etc). Thus it is important to take into account the health status of animal and the nature of the diet. Better insight into the mechanism of yeasts action is a focus of future research, especially its impact on gut morphology, gut microbiology and immunity. Supplementing *Saccharomyces cerevisiae* could be a solution for persisting acidosis problems.

***Saccharomyces cerevisiae* u ishrani preživara**

Marija Pavlović, Aleksandra Tasić, Ksenija Nešić, Snežana Ivanović

Rezime

Upotreba *Saccharomyces cerevisiae* kao aditiva hrani za preživare je široko rasprostranjena i to u vidu živih ćelija ili derivata ćelijskog zida. Brojni pozitivni efekti su dokazani. *S. cerevisiae* redukuje nivo kiseonika u rumenu, favorizuje anaerobiozu i rast celulolitičkih bakterija. Takođe, promoviše konverziju laktata u piruvat i posledično stabilizuje ruminalni pH. Suplementacija obroka ćelijama ovog kvasca će smanjiti koncentraciju amonijaka, a povećati sintezu mikrobnog proteina. Ćelije kvasaca imaju sposobnost adherencije za pojedine patogene, najpre preko oligosaharadinih komponenti manan oligosahrida i β -glukana, za koje se smatra i da promovišu fagocitnu aktivnost neutrofila. Smatra se da ćelije kvasca dodate u obrok muznim kravama, povećavaju proizvodnju mleka i smanjuju broj somatskih ćelija. Zaključno, može se podvući da je upotreba *S. cerevisiae* kao aditiva u ishrani preživara opravdana, kao i da može predstavljati potencijalno rešenje za probleme acidoze mlečnih krava.

Ključne reči: ishrana, kvasci, *Saccharomyces cerevisiae*, rumen, proizvodnja mleka

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IN-VITRO ANALYSIS OF GAS PRODUCTION OF ROUGH AND JUICY FEEDS WITH FRESH AND LYOPHILIZED RUMEN FLUID

Daniela Yordanova, Georgi Kalaydzhev, StaykaLaleva, Vladimir Karabashev, Teodora Angelova, Evgeni Videv

Agricultural institute – Stara Zagora, Bulgaria
Original scientific paper

Abstract: Content of ADF, NDF, digestibility and energy value of wheat straw, alfalfa hay and maize silage, determined by fresh and lyophilized rumen fluid of Bulgarian dairy synthetic population breed (BDSP) rams were examined. We established gas production of maize silage with fresh and lyophilized rumen fluid at different incubation time. At all hours of incubation the higher gas production recorded at fresh inoculum than lyophilized. The closest values were for fresh (93.02 dmean, ml) and lyophilized (92.730 dmean, ml) inoculum at 24 h incubation. Bigger differences were observed at 48h, 72h and 120 h. The gases released were lower with the lyophilized rumen fluid by 15.5%, 13.4% and 13% at 48h, 72h and 120 h of incubation respectively. The gas production from alfalfa hay with lyophilized rumen fluid, as well as in maize silage, was lower than fresh. Biggest differences were found at 24 h - 84.41 dmean, ml. for lyophilized and 124.67 dmean, ml for fresh inoculum. The gases released were lower for lyophilized inoculum by 17.2%, 15.6% and 14.6% at 48h, 72h and 120h of incubation respectively, which is very close to the established values for maize silage. Unlike alfalfa hay and maize silage, gas production at the 24-hour of incubation of straw was higher with lyophilized rumen fluid compared to fresh - 93.55 dmean, ml and 85.77 dmean, ml respectively. At 48, 72 and 120 hours, the released amount of gases increased slightly and have close values with slight predominance at the lyophilized inoculum.

Key words: alfalfa hay, fresh rumen fluid, gas production, lyophilized rumen fluid, maize silage, wheat straw.

Introduction

Economically feasible management of resources and waste is the first factor for the sustainable development of the livestock sector and hence the production of gases (CH₄ and CO₂) released from ruminants. Raw materials from agriculture represent the most expensive investment in terms of environmental

impact, since their production is associated with high consumption of resources - water, air, energy, land (Steinfeld *et al.*, 2006; Steinfeld *et al.*, 2013, Beschta *et al.*, 2012 Henderson, 2016, McAlpine *et al.*, 2009).

On the planet an average of 25 million ruminants have been growing every year for the past 50 years (Ripple, 2013). Globally, the livestock sector is responsible for approximately 14.5% of all anthropogenic greenhouse gas emissions. Approximately 44% of these are in the form of CH₄ from intestinal fermentation, feeds and fertilizer (Gerber, *et al.*, 2013).

The aim of this study is to research the possibility of using lyophilized inoculum in comparative analyzes by in vitro methods to overcome variability resulting from seasonal differences in the feeding of cannulated ruminants.

Material and method

To be reproductive over time in vitro systems, the inoculum must respond to specific characteristics. Considering the accuracy of the gas release kinetics for prolonged incubation periods, slight inoculum variations due to host effects, nutrition, time for taking the samples and source, and sample preparation can have significant cumulative effects for gas production.

In the analysis samples of alfalfa hay and silage from several farms were included, and straw is only from the institute's farm.

In order to limit the potential mistakes for the achievement of the set goals, Bulgarian dairy synthetic population breed (BDSP) rams were included in the experiment. They were about 2-3 years of age and about the same live weight (110-115 kg).

The method for determining the carbon emission of different feed sources is by the method of analysis with the Ankom RF Gas Production System, which enables, compared to other in vitro systems, a rapid and representative analysis of an extremely complex functioning and multicomponent a system like the one in the ruminants. This innovative technique allows us to determine the degradability of feed and gas production. The gas emission measurement system consists of 50 modules equipped with pressure sensors (pressure range: - 69. 3447 kPa; resolution: 0.27 kPa; accuracy: \pm 0.1% of the measured value) connected wirelessly to a computer, each of which it has a separate ID number from 1 to 50. The production of cumulative greenhouse gases is recorded with the Excel program in tables that can easily create curves for gas production (ANKOM, Technology).

Use Medium solution - 50 ml for each feed unit. The day on which the samples will be incubated in the morning is a Reduction solution (water, sodium hydroxide, sodium sulfide) reducing solution, which together with the other

solutions is tempered in a water bath at $T = 39^{\circ} \text{C}$. Dip the carbon dioxide hose about (20 min - 30 min) into the solution, then remove and measure the pH.

Gas production data are expressed as gas produced per gram incubated dry matter (GP - ml/g incubated dry matter DM).

To obtain reliable results, each feed sample was analyzed with 3 replicates.

Data processing was performed with the software product 13, and graphic processing with excel.

Results and discussion

Coarse / hay and straw / and juicy / silage / feed are essential in feeding ruminants. Their quality depends on the harvesting phase, technology and storage method.

The results in Table 1 showed the chemical composition of alfalfa hay, wheat straw and maize silage. According to the obtained results, the tested feeds are of very good quality. The crude protein content in the maize silage is 2.06% in initial moisture and 8.22% in absolute dry condition - specific values for the milky-waxy phase of maize grow, which is optimal for the preparation of silage. The level of raw fats and minerals are typical of maize silage at milky-waxy phase of grow – 2 – 7% (farmwest.com).

The crude protein content of alfalfa hay is 13.44% in initial moisture and 16.22% in absolute dry state. The values are typical of good quality hay prepared in the start-up phase until full flowering.

According to the established level of crude protein and other chemical composition, the quality of straw is considered good.

Table 1. Chemical composition of alfalfahay, wheat straw and maize silage.

№	Sample	Dry mater %	at initial moisture			at absolute dry state		
			Crude protein %	Crude fat %	Crude ash %	Crude protein %	Crude fat %	Crude ash %
1	Wheat straw	89.64	3.49	1.10	6.83	3.89	1.23	7.32
2	Alfalfa hay	82.85	13.44	2.47	8.35	16.22	2.98	9.28
3	Maize silage	22.83	2.06	0.85	1.76	8.22	3.71	7.05

Digestibility and energy value studies are vital for the balanced feeding of ruminants, but they require considerable resources in terms of labor, feed, animals and time in in-situ methodologies. In addition, the high associated costs, limited analytical capacity and inability to evaluate many types of feeds necessitated the use of in vitro methodologies in assessing the digestibility and energy value of feeds.

Acid-detergent fibers and neutral-detergent fibers are important indicators of the quality of feeds consumed by ruminants. They are an indicator of the volume of ration and its energy value that an animal in different category and physiological condition must absorb.

Table 2 shows the content of ADF, NDF, digestibility and energy value of alfalfa hay, wheat straw and maize silage treated with fresh and lyophilized rumen fluid. The digestibility values for wheat straw (68.72%), alfalfa hay (62.34%) and maize silage (29.48%) were lower for lyophilized rumen fluid compared to fresh inoculum (64.92%, 58.42%, 26.94%, respectively).

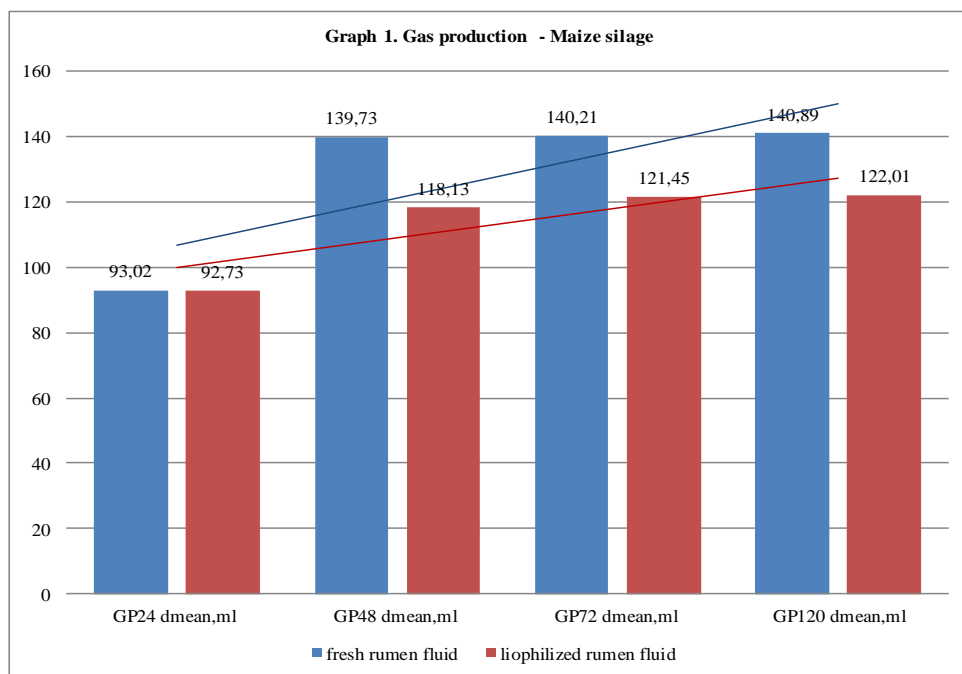
However, these differences are unreliable. The energy value for the lyophilized inoculum was higher (5.904 ME) than the fresh fluid for maize silage (5.676 ME). In the case of alfalfa hay higher value was obtained with fresh rumen fluid (6.813 ME). compared with lyophilized (5,637 ME). Established values for silage do not differ.

Table 2. ADF, NDF, digestibility and energyvalue of alfalfahay, straw and maize silage.

№	SAMPLE	ADF, %	NDF, %	DIGESTIBILITY, %		ME	
				FRESH RUMEN FLUID	LIOPHILIZED RUMEN FLUID	FRESH RUMEN FLUID	LIOPHILIZED RUMEN FLUID
1	Wheat straw	23.127	38.595	68.722	64.920	5.888	5.880
2	Alfalfa hay	37.893	46.823	62.336	58.424	6.813	5.637
	Maize silage	52.071	74.725	29.481	26.936	5.676	5.904

The following graph 1 presents the gas production data for maize silage with fresh and lyophilized rumen fluid and different incubation time.

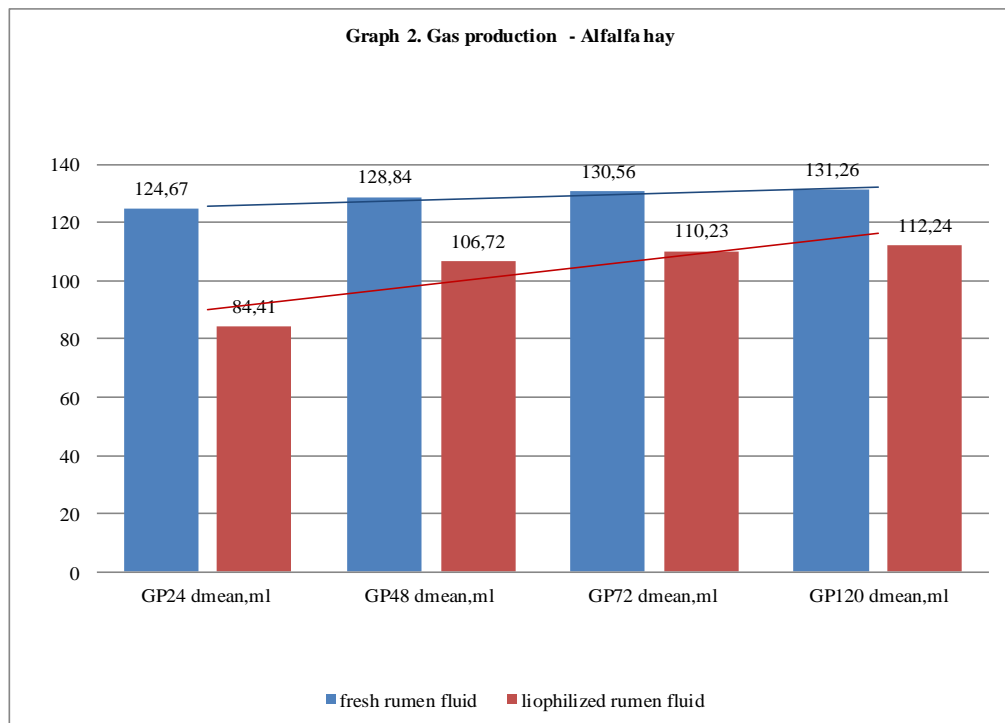
At all hours of incubation, we have a higher gas production at fresh inoculum than lyophilized. The closest values were for fresh and lyophilized inoculum at 24 h incubation - 93.020 and 92.730 dmean, ml respectively. Bigger differences were observed at 48h, 72h and 120 h. The gases released were less with the lyophilized rumen fluid by 15.5%, 13.4% and 13% respectively at 48h, 72h and 120 h of incubation.



Graph 2 shows data for gas production of alfalfa hay with fresh and lyophilized rumen fluid and different incubation times. The gas production from alfalfa hay with lyophilized rumen fluid, as well as in maize silage, was lower than fresh. Biggest differences were found at 24 h., - 84.41 dmean, ml. for lyophilized and 124.67 dmean, ml for fresh inoculum. The gases released were less for lyophilized inoculum by 17.2%, 15.6% and 14.6% respectively at 48h, 72h and 120h of incubation, which is very close to the established values for maize silage.

Graph 3 shows the results of wheat straw analysis with fresh and lyophilized rumen fluid and different incubation time.

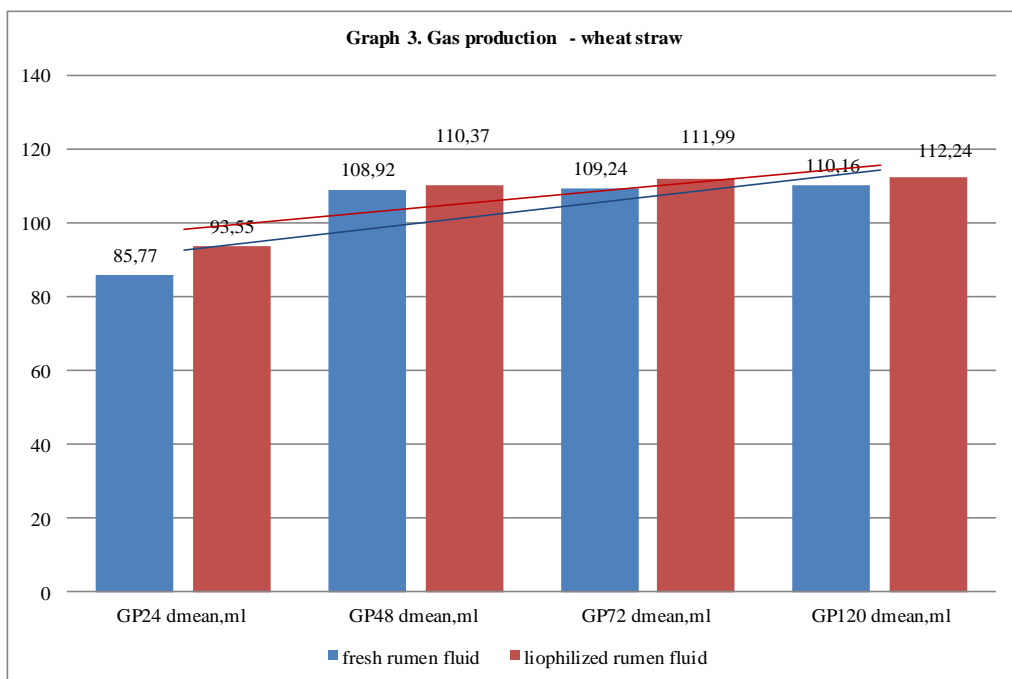
Unlike alfalfa hay and maize silage, gas production at the 24-hour of incubation of straw was higher with lyophilized rumen fluid compared to fresh



93.55 dmean, ml and 85.77 dmean, ml respectively. At 48, 72 and 120 hours, the released amount of gases increased slightly and have close values with slight predominance at the lyophilized inoculum.

Conclusion

1. In the analysis of alfalfa hay and maize silage, higher levels of gas production were found at 24, 48, 72 and 120 h of incubation levels using fresh inoculum compared to lyophilized.
2. At wheat straw analysis, higher gas production levels were



detected at 24, 48, 72 and 120 h of incubation levels using fresh inoculum compared to lyophilized.

3. Differences in gas production at fresh and lyophilized inoculum were insignificant and unreliable.

In vitro analiza proizvodnje gasa u grubim i slatkim hranivima sa svežom i liofilizovanom tečnošću rumena

Daniela Yordanova, Georgi Kalaydzhiiev, Stayka Laleva, Vladimir Karabashev, Teodora Angelova, Evgeni Videv

Rezime

Ispitan je sadržaj ADF-a, NDF-a, svarljivost i energetska vrednost sena lucerke, pšenične slame i silaže kukuruza, utvrđene svežom i liofilizovanom tečnošću rumena. Uspostavili smo proizvodnju sena lucerke sa svežom i liofilizovanom tečnošću rumena i u različitom vremenu inkubacije. Za sve periode inkubacije, imamo veću proizvodnju gasa u svežem inokulumu od liofilizovanog. Najbliže vrednosti bile su za sveži i liofilizovani inokulum pri 24 h inkubaciji – 93,020 i 92,730 dmean, ml respektivno. Veće razlike su primećene tokom 48h, 72h i 120h. Oslobođeni gasovi bili su manji sa liofilizovanom tečnošću rumena za 15,5%, 13,4% i 13%, inkubacijom 48h, 72h i 120h. Proizvodnja gasa iz sena lucerke sa liofilizovanom tečnošću rumena, kao i u silazi kukuruza, bila je manja nego sa svežom. Najveće razlike su utvrđene tokom 24 sata, - 84,41 dmean, ml za liofilizovani i 124,67 dmean, ml za sveži inokulum. Oslobođeni gasovi bili su manji za liofilizirani inokulum za 17,2%, 15,6% i 14,6% respektivno, u 48h, 72h i 120h inkubacije, što je vrlo blizu utvrđenim vrednostima za silažu kukuruza. 24-satna inkubacija slame bila je veća sa liofilizovanom tečnošću rumena u poređenju sa svežom - 93,55 dmean, ml i 85,77 dmean, ml respektivno. Za 48, 72 i 120 sati, oslobođena količina gasova blago se povećala i ima bliske vrednosti sa blagom dominacijom u liofilizovanom inokulumu.

Ključne reči: seno lucerke, sveža tečnost rumena, proizvodnja gasa, liofilizovana tečnost, silaža kukuruza, pšenična slama.

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COMPARING THE CHEMICAL COMPOSITION OF THE LAMB MEAT OF VARIOUS NATIVE BREEDS

Marzena Zająć¹, Joanna Tkaczewska¹, Piotr Kulawik¹, Paulina Guzik¹,
Bronisław Borys², Łukasz Migdał³, Anna Migdał⁴, Władysław Migdał¹

¹Department of Animal Product Technology, Faculty of Food Technology, University of Agriculture in Krakow, ul. Balicka 122, 31-149 Kraków, Poland

²National Research Institute of Animal Production, Experiment Station Kołuda Wielka, 88-160 Janikowo, Poland

³Department of Genetics and Animal Breeding, Faculty of Animal Science, University of Agriculture in Krakow, al. Mickiewicza 24/28, 30-059 Kraków, Poland

⁴Institute of Veterinary Sciences, Faculty of Animal Science, University of Agriculture in Krakow, al. Mickiewicza 24/28, 30-059 Kraków, Poland

Corresponding author: Władysław Migdał, wladyslaw.migdal@urk.edu.pl

Original scientific paper

Abstract: High quality lamb meat production depends on many factors, especially on the production technology including: genotype, gender, feeding system, standard slaughter weight (also associated with age), the breed and breeding conditions. Those factors affect the production yields, profitability, quality and the dietetic value of meat. The aim of this work was to compare the chemical composition of lamb meat coming from native breeds of sheep bred in two different feeding systems. The meat from native sheep bred in the Institute IZ PIB ZD Kołuda Wielka contains less water compared to the traditional feeding system. The original concentrated feed composed in the Institute IZ PIB ZD Kołuda Wielka positively affected the meat quality regarding the chemical composition. Lamb meat from native breeds can be treated as a high quality and nutritious food because of the high protein and mineral content with relatively low fat content.

Key words: lamb, native breeds, feeding systems, meat, chemical composition

Introduction

The consumers look for good quality foods which are tasty and healthy. That is why lamb meat can be a good alternative for the most popular meats like poultry, pork and beef. Thanks to the presence of bioactive compounds in lamb meat we can call it functional food. It contains high biological quality proteins, L-

carnitine, conjugated linoleic acid (CLA), micro- and macro elements. Lamb meat is recommended as a highly nutritious food, especially for children, convalescents and elderly. Sheep breeds can be divided into various groups depending on the quality of received raw material, tail length, thickness and color of wool or the region from which the animals come (mountains, plains). The other type of grouping is on primitive (domestic) breeds like Romanov breed or noble breeds like Merino (*Grzeškowiak et al., 2003*). The main breeding direction in Poland is milk production which results from the environmental and economic conditions. The region of Warmia and Mazury is predestinated to this kind of sheep utilization because of the agricultural conditions – large amounts of open grasslands, perfect for grazing sheep (*Milewski, 2010*).

High quality lamb meat production depends on many factors, especially on the production technology including: genotype, gender, feeding system, standard slaughter weight (also associated with age), the breed and breeding conditions. Those factors affect the production yields, profitability, quality and the dietetic value of meat (*Borys and Borys, 2001; Borys and Pisulewski, 2001; Szewczuk et al., 2009*). To improve the nutritional value of lamb meat some additives like herbs, linseed or rapeseed are incorporated to feeds. Adding raw materials rich in omega 3 fatty acids improves meat's value by increasing the polyunsaturated fatty acid (PUFA) content (*Junkuszew, 2004; Borkowska, 2011*).

The aim of this work was to compare the chemical composition of lamb meat coming from native breeds of sheep bred in two different feeding systems.

Material and Methods

Material

The research material, used for the analysis, was lamb meat and meat products, coming from 8 native sheep breeds fattened by the system used in their holding regions (GM) and an original method aimed to obtain beneficial health-related modifications in the field of meat and meat quality (KW).

Fattening methods used in maternity farms (GM)

The Uhruska sheep (UHR):

The lambs intended for slaughter were fattened with a mix of cereal grits with mineral and vitamin supplementation, silage, grass hay and a hay made of mix of grasses and clover. The lambs were fed without any nutritional restrictions.

Pomeranian sheep (POM):

The lambs raised for slaughter, after weaning, were fattened with hay and whole barley grains without restrictions, as well as with a complete feed for calves and lambs (0.5 - 0.6 kg / day / pc). The lambs were kept in alcoves, without grazing in the pasture.

Black-headed sheep (CZGL):

During summer feeding (from May to October) lambs were kept in pasture, while during winter they were fed hay, straw and a feed composed mainly of oats and barley with the addition of lupine or soy (up to 33%). The feed was enriched with vitamins and chalk pasture. The lambs were fed without any nutritional restrictions.

Old Polish Merino sheep (MST):

Lambs intended for slaughter were fattened with feed based on specialized concentrates, cereal grits, soy meal and dried beet pulp, as well as hay which they received without restrictions. The lambs were kept in alcoves, without grazing in the pasture.

Wielkopolska sheep (WLKP):

The experimental lambs were kept in alcoves, with no access to the pasture, remaining with mothers until the age of 90 days. During the fattening period, animals were fed *ad libitum* with a mixture consisting of crushed wheat grains, oats and triticale, soy meal, corn meal and a supplementary mixture for sheep (2%). The lambs also received hay, without restrictions.

Cakiel podhalański (CKP):

Lambs assigned for slaughter, after weaning on the 100th day, grazed on pastures and fed small amounts of bran.

Świniarka (SWIN):

The lambs assigned for slaughter were grazed on pastures and fed a small amount of a mixture of cereal grits.

Wrzosówka (WRZOS):

Lambs intended for slaughter were weaned at 4 months of age and then grazed on pastures.

Fattening methods used in IZ PIB ZD Kohuda Wielka (KW)

The lambs of all compared breeds were fed a mixture containing 15% rapeseed cake, 15% dried corn stool and 5% flax seed in the amount of 3% of lamb body weight and grass hay, which they received without restrictions. The Uhruska sheep,

Pomeranian sheep, Black-headed sheep, Old Polish Merino sheep, Wielkopolska sheep and Cakiel podhalański were fattened to obtain an average weight of 35-40 kg, and Świniarka and Wrzosówka to achieve an average weight of 30-35 kg.

After reaching the appropriate weight, the lambs were slaughtered and transported to the laboratory. All the analyses were conducted on the *longissimus dorsi lumborum* muscle.

Methods

Dry matter, total proteins, fat and ash were measured according to AOAC (2000). The following items were estimated in the raw meat samples:

- water content according to the standard (PN-ISO 1442:2000),
- fat content according to the standard (PN-ISO 1444:2000),
- protein content by Kjeldahl method (PN-75/A-04018),
- total ash content according to the standard (PN-ISO 936:2000),

Results and Discussion

The results of the proximate analysis of the lamb meat of various breeds and feeding systems are presented in Table 1.

The highest dry matter content was noted for the meat from Świniarka KW and Old Polish Merino KW (26.56% and 27.05% respectively), and the lowest in Wrzosówka GM and Cakiel GM (22.39% and 19.61% respectively). It can be noted, that in most of the breeds (MST, WLKP, CZGL, WRZOS, SWIN, POM) the industrial feeding system led to the higher level of dry matter in the analysed meat. The differences were negligible except from MST breed in which the difference in the dry matter content was over 3 percentage points. The industrial feeding is concentrated on the weight gain along with low feed intake. The feed must be balanced, so that the animal gets adequate proportions of nutrients which may lead to higher dry matter content. In the same breeds the fat and protein content was higher. The fat content influences the dry matter content significantly. The highest protein content was detected in Black-headed breed and the lowest in Cakiel podhalański.

Table 1. The chemical composition of lamb meat depending on the breed and type of feeding system

Breeds of lambs	Feeding systems	Dry mass [%]	Protein [%]	Fat [%]	Ash [%]
Uhruska sheep UHR	KW	23.47 ± 0.15	20.66 ± 0.48	1.96 ± 0.2	1.10 ± 0.01
	GM	23.91 ± 0.07	20.66 ± 0.11	2.23 ± 0.18	1.11 ± 0.02
Cakiel podhalański CKP	KW	20.65 ± 0.33	16.36 ± 3.46	2.22 ± 0.25	1.10 ± 0.07
	GM	19.61 ± 0.15	15.98 ± 0.71	1.76 ± 0.24	1.16 ± 0.06
Pomeranian sheep POM	KW	24.37 ± 0.55	20.83 ± 2.66	1.82 ± 0.49	1.14 ± 0.08
	GM	23.39 ± 0.15	19.06 ± 0.12	1.27 ± 0.1	1.34 ± 0.09
Świniarka SWIN	KW	26.56 ± 0.12	20.48 ± 0.23	5.46 ± 2.38	1.07 ± 0.08
	GM	23.95 ± 0.05	19.27 ± 3.86	4.60 ± 0.13	1.05 ± 0.05
Wrzosówka WRZOS	KW	24.86 ± 1.18	21.78 ± 1.06	2.10 ± 0.41	1.20 ± 0.18
	GM	22.39 ± 0.34	19.12 ± 1.75	1.74 ± 0.08	1.10 ± 0.03
Black-headed sheep CZGL	KW	24.69 ± 0.67	21.17 ± 1.98	2.22 ± 0.25	0.98 ± 0.09
	GM	24.08 ± 0.25	22.16 ± 1.75	1.76 ± 0.24	1.15 ± 0.04
Old Polish Merino sheep MST	KW	27.05 ± 2.69	20.84 ± 1.01	5.01 ± 0.23	1.23 ± 0.08
	GM	23.90 ± 2.98	19.56 ± 4.50	4.62 ± 0.63	1.11 ± 0.01
Wielkopolska sheep WLKP	KW	25.23 ± 0.69	20.25 ± 2.75	7.47 ± 0.60	1.09 ± 0.01
	GM	25.58 ± 0.19	18.98 ± 1.89	9.14 ± 0.38	1.05 ± 0.07

Mean values ± standard deviation values

GM - fattening methods used in maternity farms

KW - fattening methods used in IZ PIB ZD Kołuda Wielka

The quality traits – including chemical composition – may be influenced by sheep genotype. *Juárez et al. (2009)* analyzing the meat from merino and churra breeds slaughtered at the weight of 20 kg obtained much higher protein and intramuscular fat content in merino breed. The highest fat content obtained in these studies was observed in Wielkopolska breed both from KW (7.47%) and GM (9.14%) feeding systems and also in Świniarka breed from KW (5.46%) feeding system. The lowest fat content was noted in Pomorska breed from GM feeding system (1.27%).

The level of fat in lamb meat depends both on the feeding method and the breed and it is possible to modify those factors. According to *Grześkowiak (2003)* the optimal amount of intramuscular fat in muscle tissue should be in the range of 1.5-2.5% to obtain the best sensory quality. The results in that range were obtained by *Borys and Borys (2002)* in lamb meat of various breeds. However, in the

presented studies the amount of fat in most of the breeds was higher. According to *Kędzior (2005)* the changes of chemical components' proportions which are observed during growing period and the body mass increase, are associated mainly with the subcutaneous fat increase. It is especially noticeable in bigger animals in which the muscle mass content decreases at the same time.

Gruszecki et al. (2001) noted that lambs fed in alcoves are growing faster and that they obtain the body weight of 30 kg 20 days earlier compared to the animals fed on pastures. Moreover, they have better body conformation, but higher subcutaneous fat content. Pasture feeding resulted in higher muscle content and lower fat content compared with the meat of animals fed the concentrated feed (*Ripoll et al., 2010*). It can be concluded that the intensive breeding causes relatively fast fat accumulation while at slower feeding intensity the muscling is higher at the same body weight.

The highest ash content was noted in Old Polish Merino from KW feeding system. The other breeds had comparable ash content ranging from 0.98% in Czarnogłówka fed in KW system while the highest in GM feeding system was 1.34% in Pomorska. It can be noted that meat from Wrzosówka meat from industrial feeding system contained significantly higher ash content than the same breed from traditional feeding system. That type of breed has very low feeding and breeding requirements and the animals easily adapt to variable climate or environmental conditions (*Grześkowiak et al., 2011*). Probably these features are the cause of better feed conversion.

Conclusions

Lamb meat from native breeds can be treated as a high quality and nutritious food because of the high protein and mineral content with relatively low fat content. The meat from native sheep bred in the Institute IZ PIB ZD Kołuda Wielka contains less water compared to the traditional feeding system. The original concentrated feed composed in the Institute IZ PIB ZD Kołuda Wielka positively affected the meat quality regarding the chemical composition.

Poređenje hemijskog sastava jagnjećeg mesa različitih autohtonih rasa

Marzena Zajac, Joanna Tkaczewska, Piotr Kulawik, Paulina Guzik, Bronisław Borys, Władysław Migdał

Rezime

Proizvodnja visokokvalitetnog janječeg mesa zavisi od mnogih faktora, posebno od tehnologije proizvodnje, uključujući: genotip, pol, sistem hranjenja, standardnu težinu na klanju (takođe povezanu sa godinama), rasu i uslove uzgoja. Ti faktori utiču na proizvodne prinose, profitabilnost, kvalitet i hranljivu vrednost mesa. Cilj ovog rada bio je upoređivanje hemijskog sastava jagnječeg mesa koje potiče od autohtonih rasa ovaca uzgajanih u dva različita sistema ishrane. Meso domaćih ovaca uzgajanih u Institutu IZ PIB ZD Kołuda Wielka sadrži manje vode u poređenju s tradicionalnim sistemom hranjenja. Originalna koncentrovana hrana sastavljena u Institutu IZ PIB ZD Kołuda Wielka pozitivno je uticala na kvalitet mesa u pogledu hemijskog sastava. Jagnjeće meso autohtonih rasa može se tretirati kao visokokvalitetna i hranljiva hrana zbog visokog sadržaja proteina i minerala sa relativno niskim sadržajem masti.

Ključne reči: jagnjetina, autohtone rase, sistemi ishrane, meso, hemijski sastav

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MEAT QUALITY OF BROILERS IN AN EXTENDED FATTENING PERIOD

Vladimir Dasković¹, Snežana Bogosavljević-Bošković¹, Lidija Perić², Zdenka Škrbić³, Simeon Rakonjac¹, Veselin Petričević³

¹ Faculty of Agronomy, University of Kragujevac, Cara Dušana 34, 32000 Čačak, Serbia

² Faculty of Agriculture, University of Novi Sad, trg Dositeja Obradovića 8, 21101 Novi Sad, Serbia

³ Institute for Animal Husbandry, Autoput 16, P. Fah 23, 11080, Belgrade–Zemun, Serbia

Corresponding author: Vladimir Dasković, vladasko@kg.ac.rs

Original scientific paper

Abstract: This study evaluates the effect of protease-supplemented diets containing different crude protein levels, sex and length of the fattening period on the weight and percentage yield of primal cuts in Cobb 500 broilers. Chickens were fed maize-and-soybean-based diets. Fattening periods lasted 49 and 63 days. The results showed that the recommended crude protein reduction by 4% in diets supplemented with 0.2% protease had no effect on the weight and percentage yield of primal cuts ($P>0.05$), while the effect of broiler sex and slaughter age on the studied carcass quality parameters was significant ($P<0.05$).

Keywords: broilers, protease, sex, fattening period

Introduction

In the last several decades, broiler chickens have increasingly been reared in non-standard i.e. non-commercial systems. Modifications of conventional commercial production systems involve using other hybrids (medium growing hybrids, slow growing hybrids, fattening breeds of hens), reducing stocking density (often allowing access to an outdoor grass range) and feeding new formulas (particularly to improve nutrient digestibility, health and immunity, etc.). As explained by broiler farmers and experts, major reasons for these modifications include the improvement of farm animal welfare and poultry meat quality, and reduction of environmental pollution.

Numerous researchers have investigated alternative broiler fattening systems from the viewpoint of: genotype (Škrbić *et al.* 2007, Meluzzi *et al.* 2009, Madeira *et al.* 2011), stocking density (Beg *et al.* 2011), fattening period (Mitrović *et al.* 2010, Bogosavljević-Bošković *et al.* 2011), dietary protein reduction

(Horniakova and Abas, 2009), and enzyme supplementation (Dosković *et al.* 2017, Vojtěch *et al.* 2016, Mohammadigheisar and Kim 2018).

This study evaluates the quality of meat from fast growing Cobb 500 male and female broiler chickens receiving protease supplemented diets over an extended fattening period (49 and 63 days). The weights and percentage yields of primal cuts were used as meat quality parameters.

Materials and Methods

The broiler feeding trial lasted 63 days. The Cobb 500 broiler chickens used as the experimental material were allocated to 2 feeding groups: a control group – C (100 broilers, a standard diet containing maize and soybean meal) and an experimental group – E-1 (100 broilers, a diet reduced in crude protein by 4% and supplemented with 0.2% Ronozyme ProAct protease).

On days 49 and 63 of the feeding trial, 10 males and 10 females were randomly selected from each group, slaughtered and processed (*Commission Regulation (EC) No. 543/2008*), and their carcasses were weighed and dissected into primal cuts i.e. breasts, thighs, drumsticks, wings, back and pelvis. The weights of primal cuts were used for the calculation of their percentage yields in the dressed carcass.

Data sets were statistically analysed by a three-way analysis of variance in a 2 x 2 x 2 design (2 diets, 2 fattening periods – FP, 2 sexes), F-test and LSD test ($P < 0.05$) using *Statistica for Windows Release 6.0 (1995)*.

Results and Discussion

Table 1 shows the weights of primal cuts of male and female broilers at 49 and 63 days of age across experimental groups.

The statistical analysis of the data on the weights of primal cuts of broilers shows similar responses of broilers to dietary treatments given their approximately equal weights of primal carcass cuts ($P > 0.05$). Extended fattening led to an increase in body weight and, hence, weight of primal cuts ($P < 0.05$). Moreover, males had greater carcass weights and, hence, greater weights of primal cuts ($P < 0.05$). Nikolova and Pavlovski (2009) also determined that broiler sex and slaughter age of the same hybrid (Cobb 500) broilers had a significant effect on breast, drumstick and thigh weights. Somewhat smaller weights of primal cuts in Cobb 500 broilers at 49 days of age were reported by Abera *et al.* (2017), who also found a significant effect of sex on breast and thigh weights, and an effect of fattening period (49 and 56 days) on the weights of drumsticks, thighs and wings.

Table 1. Weights of primal cuts across treatments, gr

Diet	Fattening period, days	Sex		Breast	Dumsticks	Thighs	Wings	Back	Pelvis
C	49	♂	\bar{x}	856.3 ^{bcd}	361.9 ^b	412.9 ^b	285.4 ^b	304.8 ^{bc}	272.8 ^b
			Sd	43.7	27.9	23.5	15.5	23.2	18.3
		♀	\bar{x}	741.1 ^d	290.9 ^c	341.8 ^d	238.3 ^c	262.3 ^d	219.6 ^c
			Sd	65.7	22.0	27.5	9.8	28.1	14.3
	63	♂	\bar{x}	1085.9 ^a	435.9 ^a	537.5 ^a	337.7 ^a	379.3 ^a	328.1 ^a
			Sd	209.8	35.4	43.9	27.1	23.7	27.7
		♀	\bar{x}	951.5 ^{ab}	332.4 ^b	406.5 ^b	257.1 ^c	306.8 ^{bc}	273.3 ^b
			Sd	141.1	33.3	54.1	11.4	22.3	24.6
E-1	49	♂	\bar{x}	803.8 ^{bcd}	343.7 ^b	397.3 ^{bc}	275.1 ^b	317.9 ^b	268.5 ^b
			Sd	73.11	11.9	29.7	11.9	24.1	22.1
		♀	\bar{x}	778.9 ^{cd}	281.4 ^c	347.3 ^{cd}	229.2 ^c	231.6 ^c	221.4 ^c
			Sd	109.2	15.6	25.2	15.6	31.4	16.9
	63	♂	\bar{x}	1110.7 ^a	426.6 ^a	516.4 ^a	329.1 ^a	378.0 ^a	328.9 ^a
			Sd	163.3	38.2	40.9	25.7	43.2	32.7
		♀	\bar{x}	968.0 ^{ab}	332.6 ^b	406.4 ^b	275.4 ^b	297.9 ^c	279.0 ^b
			Sd	145.7	21.1	36.2	22.7	27.1	23.5
p-value									
Source of variation				ns	ns	ns	ns	ns	ns
Diet				ns	ns	ns	ns	ns	ns
Fattening period				P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05
Sex				P<0.05	P<0.05	P<0.05	P<0.05	P<0.05	P<0.05
Diet x fattening period				ns	ns	ns	ns	ns	ns
Diet x sex				ns	ns	ns	ns	ns	ns
Fattening period x sex				ns	P<0.05	P<0.05	P<0.05	ns	ns
Diet x fattening period x sex				ns	ns	ns	ns	ns	ns

a-e Means followed by different superscript letters within columns differ significantly (P<0.05)

Table 2 presents the percentage yields of primal cuts in the dressed carcasses of broilers of different age and sex groups.

Table 2. Percentage yield of primal carcass cuts of broilers on days 49 and 63 of the fattening trial, %

Diet	Fattening period, days	Sex		Breast	Drumsticks	Thighs	Wings	Back	Pelvis
C	49	♂	\bar{x}	33.17 ^{bc}	14.01 ^a	15.96 ^{ab}	11.06 ^a	11.80 ^{ab}	10.57
			Sd	1.31	0.83	0.67	0.53	0.79	0.67
		♀	\bar{x}	34.03 ^{abc}	13.38 ^{ab}	15.70 ^{ab}	10.97 ^a	12.03 ^{ab}	10.11
			Sd	1.80	0.83	0.70	0.55	0.84	0.68
	63	♂	\bar{x}	33.60 ^{abc}	13.60 ^{ab}	16.78 ^a	10.53 ^{ab}	11.88 ^{ab}	10.23
			Sd	4.75	1.16	1.50	0.83	1.38	0.83
		♀	\bar{x}	35.75 ^{ab}	12.55 ^b	15.31 ^{ab}	9.75 ^b	11.62 ^{ab}	10.32
			Sd	2.66	0.79	1.19	0.80	0.85	0.57
E-1	49	♂	\bar{x}	32.09 ^c	13.75 ^a	15.87 ^{ab}	11.00 ^a	12.70 ^a	10.74
			Sd	1.90	0.42	0.66	0.26	0.63	0.88
		♀	\bar{x}	35.77 ^{ab}	13.00 ^{ab}	16.05 ^{ab}	10.58 ^{ab}	10.66 ^b	10.19
			Sd	4.02	1.11	1.55	0.85	1.34	0.52
	63	♂	\bar{x}	34.61 ^{abc}	13.36 ^{ab}	16.18 ^{ab}	10.32 ^{ab}	11.82 ^{ab}	10.33
			Sd	2.75	0.66	0.93	0.60	0.95	1.06
		♀	\bar{x}	36.20 ^a	12.51 ^b	15.27 ^b	10.37 ^{ab}	11.23 ^b	10.51
			Sd	3.21	0.52	0.76	0.84	1.04	0.81
p-value									
Source of variation									
Diet				ns	ns	ns	ns	ns	ns
Fattening period				ns	P<0.05	ns	P<0.05	ns	ns
Sex				P<0.05	P<0.05	P<0.05	ns	P<0.05	ns
Diet x fattening period				ns	ns	ns	ns	ns	ns
Diet x sex				ns	ns	ns	ns	ns	ns
Fattening period x sex				ns	ns	P<0.05	ns	ns	ns
Diet x fattening period x sex				ns	ns	ns	ns	P<0.05	ns

^{a-c} Means followed by different superscript letters within columns differ significantly (P<0.05)

As shown by the results in Table 2, broiler diet had no effect on the percentage yields of primal cuts (breasts, drumsticks, thighs, wings, back, pelvis) (P>0.05). Similarly, crude protein reduction in broiler diet supplemented with protease (0.05% and 0.1% Ronozyme Pro Act) had no effect on some carcass quality traits (breast, liver and heart yields) in a study by *Mohammadigheisar and Kim (2018)*. Significant differences were observed in the percentage yields of some primal cuts between male and female broilers, as well as between different broiler age groups (P<0.05). Females had greater breast yields and lower drumstick yields compared to males (P<0.05), and their wing yields in the dressed carcass at 49 days of the fattening trial were higher than in male broilers of 63 days of age (P<0.05),

whereas the percentage yields of the other primal cuts did not significantly differ between the sexes and slaughter age groups ($P>0.05$). In 49-day-old Cobb 500 broilers, *Fomentini et al. (2016)* determined the percentage yields of 36.9% for breasts, 15% for drumsticks and 15.8% for thighs. The results reported by *Moreira et al. (2003)* were similar to the present data i.e. the authors found no significant difference in the percentage yields of breasts, thighs and drumsticks across slaughter age and sex groups. In Cobb 500 broilers at 49 days of age, *Fernandes et al. (2013)* obtained the percentage yields of 23.2% for breasts, 33.98% for thighs and drumsticks, and 10.95% for wings, and found no dependence of the percentage yields of breasts, thighs, drumsticks and wings on sex and age, while interactions often occur as the result of the effect of hybrid, sex and slaughter age. *Nikolova and Pavlovski (2009)* stressed that sex affects the percentage yield of thighs (lower values were found in females than in males), while having no effect on breast and drumstick yields, and that extended fattening leads to an increase in the percentage yields of the three primal cuts. In contrast, *Bogosavljević-Bošković et al. (2011)* found no effect of slaughter age on the percentage yields of primal cuts in Cobb 500 broilers, but observed significant differences between the sexes in the percentage yields of breasts, thighs and drumsticks (females had higher breast yields, while male broilers had higher thigh and drumstick yields).

Conclusion

The results of the present research indicate that:

- protease supplementation (0.2%) of broiler diets containing crude protein levels reduced by 4% had no effect on the weights and percentage yields of primal cuts (breasts, thighs, drumsticks, wings, back, pelvis),
- significant differences ($P<0.05$) were observed between male and female broilers in primal cut weights (higher values found in males) and breast and drumstick yields (females had greater breast yields and lower drumstick yields, $P<0.05$),
- the 14-day extension of the fattening period (from 49 to 63 days) resulted in increased weights of primal cuts ($P<0.05$), whereas the percentage yields of primal cuts in the dressed carcass showed no significant differences ($P>0.05$) between the two age groups of broilers (only the percentage yield of wings at 49 days of the feeding trial was higher than in broilers aged 63 days, $P<0.05$).

Kvalitet mesa brojlera iz produženog trajanja tova

Vladimir Dosković, Snežana Bogosavljević-Bošković, Lidija Perić, Zdenka Škrbić, Simeon Rakonjac, Veselin Petričević

Rezime

U radu je ispitivan efekat hrane sa različitim nivoom sirovih proteina, uz dodatak enzima protease, pola pilića i vremena trajanja tova na masu i udeo osnovnih delova trupa brojlera hibrida Cobb 500. Pilići su hranjeni potpunim smešama na bazi kukuruza i sojine sačme. Tov pilića trajao je 49 i 63.dana. Na osnovu dobijenih rezultata zaključeno je da predložena supstitucija smanjenja sadržaja sirovih proteina za 4% u odnosu na standardni obrok, uz dodatak 0,2% enzima protease, nije uticala na masu i udeo osnovnih delova trupa ($P>0,05$), kao i da se ispoljio značajan efekat pola pilića i vremena klanja pilića na ispitivane parametre kvaliteta trupa brojlera ($P<0,05$).

Ključne reči: brojleri, proteaza, pol, trajanje tova.

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EGG QUALITY OF COMMERCIAL LAYER HYBRID KEPT IN DIFFERENT HOUSING SYSTEMS

Zdenka Škrbić¹, Miloš Lukić¹, Veselin Petričević¹, Snežana Bogosavljević-Bošković², Simeon Rakonjac², Vladimir Dasković², Nataša Tolimir³

¹Institute for Animal Husbandry, Autoput 16, 11080, Belgrade-Zemun, Republic of Serbia

²Faculty of Agronomy, University of Kragujevac, Cara Dušana 34, 32000 Čačak, Republic of Serbia

³Institute of Science Application in Agriculture, Bulevar Despota Stefana 68b, 11000, Belgrade, Republic of Serbia

Corresponding author: Zdenka Škrbić, zskrbic@istocar.bg.ac.rs

Original scientific paper

Abstract: Egg quality parameters of hybrid layers were analyzed by comparative testing of conventional cage and alternative housing systems: floor pen and mobile cages on the pasture. The analysis of the quality of eggs from the conventional cage and floor pen housing system was carried out at the age of 42 to 44 weeks, and from the conventional cage and the mobile cage on pasture, in the period from 51 to 53 weeks of age. Each trial was organized in 3 repetitions in the 3 seven-day periods and 30 eggs per repetition, i.e. 90 per housing system were analyzed. The quality of 360 eggs in total was analyzed. The initial egg quality was studied based on the properties of the external and internal quality of eggs and eggshell quality. In the conditions of the same genetic of laying hens, the parameters of the egg quality that were significantly affected by the housing system are the egg weight, certain properties of eggshell quality (cleanliness, colour and weight), the ratio of the albumen and yolk, and the yolk colour. Significantly higher egg weight and a lower albumen to egg yolk ratio were recorded in eggs from alternative housing systems compared to the conventional cage system. Eggs from the floor system had a darker and dirtier egg shell compared to the conventional cage system. The eggshell weight and the yolk colour were significantly higher in eggs from mobile cages.

Key words: layers, egg quality, housing systems

Introduction

Changes in the housing system for laying hens is implied by the necessity for improvement of the conditions for the welfare of layers, and the expected

legislation on permitted housing systems in order to harmonize with the adopted principles contained in the EU Regulation (*Council Directive 1999/74/EC*). There is a great deal of consumer pressure imposing more natural production conditions as an imperative for a better product. Therefore, the topic of the effect of the housing system for layers is inspirational for researchers, not only from the aspect of hens welfare, but also from the aspect of the quality of eggs, productivity, and economy.

The effect of the housing system is based on the differences in the available space for the hens' movement and level of equipment of that space, related to the manifestation of common patterns of behaviour and welfare of hens, ambient conditions, nutrition, exposure to stress factors.

Previous studies of the effects of the housing system on the egg quality are without a clear answer to the question of the superiority of a particular system in terms of the egg quality. The reason is the inability to fully reconcile the factors important for the quality of eggs (genetic of laying hens, hen age, oviposition time, nutrition), as well as certain specificities of the investigated housing systems. The observed differences in the egg quality under the influence of the housing system relate to the egg weight, the eggshell cleanliness, the yolk colour, Haugh units (*Pavlovski et al., 2004; Pišteková et al., 2006; Rossi, 2007; Sekeroglu et al., 2008; Đukić-Stojić et al., 2009; Yilmaz Dikmen et al., 2016*). The advantage of the cage in relation to alternative housing systems in terms of the egg weight is stated by *Minelli et al. (2007)*, in terms of the albumen quality and eggshell quality, *Englmaier et al. (2014)*. *Svobodova et al. (2014)* indicate significantly higher egg weight and better albumen quality in floor compared to the cage system. By contrast, *Rizzi et al. (2006)* have not determined the differences in the individual properties of egg quality from different housing systems. Similarly, *Sekeroglu et al. (2010)* report that there are no major differences in egg quality between the floor, free range and cage systems.

Based on the above mentioned, the aim of the present study was to provide a contribution to the results of the research in this field by comparative testing of the conventional cage system and floor pens, i.e. conventional cage system and mobile cages on the pasture, from the aspect of the egg quality.

Material and Methods

The quality of the eggs from the conventional cage system was compared with the quality of the eggs from the floor pen, i.e. mobile cages on the pasture. In all three systems Tetra SL layers were used. The quality of eggs from the conventional cage and floor system of housing was tested at the age of layers of 42

to 44 weeks, and the effect of the housing in mobile cages, due to the vegetation and the adjustment of the length of the light day, was examined later, at the age of the layers of 51 to 53 weeks. Each trial was organized in 3 repetitions (3 seven-day periods) and 30 eggs per repetition were analyzed, respectively 90 per housing system. The quality of total of 360 eggs has been analyzed. The cage system of housing was in three-tier conventional cages with 4 layers per cage. The floor system was on a deep litter, in a pen area of 8m² and a stocking density of 4 layers/m². The facilities in both systems were without windows and with artificial lighting of 15 hours a day. The housing in mobile cages was on a natural pasture. The surface of the cage was 4.5 m² and the stocking density 3 layers/m². Layers were exposed to natural light for 15 hours. Nutrition of layers was consistent with their requirements at the given stage of the production cycle (*Management Guide Babolna Tetra Hybrids*) and identical for all studied housing systems. In addition, the availability of pasture for layers in mobile cages was constant. Food and water were available ad libitum. The eggs were sampled in the morning and the initial quality of the eggs was examined based on the exterior and interior egg quality properties (Pavlovski *et al.*, 1997) as well as the eggshell quality (Pavlovski and Vitorović, 1996).

Statistical data processing was done by the One-way ANOVA variance using the statistical software package STATISTICA, version 8, StatSoft, Inc. (www.statsoft.com).

Results and Discussion

The egg quality from the conventional cage and floor pen systems was statistically significantly different in terms of the egg weight, the eggshell colour, the eggshell cleanness, and the albumen yolk ratio (Table 1).

Eggs from the floor pen housing system in relation to the conventional cage system had significantly higher weight. The higher egg weight in the floor system compared to the cage system was determined by Svobodova *et al.* (2014), Pištekova *et al.* (2006). Contrary to these results is study by Đukić-Stojčić *et al.* (2009) in which they conclude that the eggs from the cage system have the greatest weight in relation to the floor system with the range and the free range system. It is difficult to discuss which factors in the housing system have caused the differences in the egg weight. In conditions of the same genetic basis and age of hens layers, and nutrition, the differences between the cage and the floor system of housing are in the environment and technological processes, and consequently, physiological stress (Holt *et al.*, 2011). The lower albumen/yolk ratio in the floor pen compared to the cage system indicates a higher proportion of egg albumen in the structure of

eggs in the floor system. Reduction of the proportion of yolk, generally in the last decades, *Flock (2019)* attributes to the selection in order to effectively convert food into egg weight. Expectedly, the eggs from the floor pen system have a dirtier shell, although there are also contrary findings (*Ahammed et al., 2014*). The eggshell colour is a genetically conditioned trait, but it differed in the examined systems of housing. However, it should be noted that the established values for the eggshell colour in both examined systems were within the standard for given layer hybrid. The differences in the eggshell colour under the influence of the housing system were determined by *Pavlovski et al. (2004)* and *Sekeroglu et al. (2010)*. Contrary to our results, *Sekeroglu et al. (2010)* have found that the eggs from the floor system have the brightest shell, while *Pavlovski et al. (2004)* have found that eggs from the cage system have a darker shell compared to eggs from the free range system.

Table 1. Quality of eggs from conventional cage and floor pen systems at the age of layers of 42-44 weeks

Parameter	Conventional cages		Floor pen		p
	Mean	SD	Mean	SD	
Egg weight, g	61.99	5.94	64.82	4.44	0.0082
Egg shape index	77.88	2.27	78.00	1.95	0.777
Shell colour, points	3.46	0.58	3.82	0.48	0.0010
Shell cleanness, points	4.64	0.78	3.94	1.20	0.0008
Shell deformation, mm	0.215	0.057	0.201	0.023	0.361
Shell weight, g	8.51	0.96	8.66	0.98	0.452
Shell thickness, mm	0.355	0.032	0.351	0.023	0.546
Shell breaking force, kg	2.74	0.66	2.76	0.35	0.886
Albumen height, mm	8.126	1.09	8.35	1.49	0.393
Albumen/Yolk ratio	2.21	0.2	2.03	0.16	<0.0001
Yolk colour, Roche	12.64	1.06	12.56	1.55	0.764
Haugh Units	88.98	6.49	90.10	5.28	0.346

The average values of egg quality parameters from the conventional cage and the system in mobile cages on the pasture are shown in Table 2.

Significant differences in egg quality parameters are determined for the egg weight, the eggshell weight, the albumen/yolk ratio and the yolk colour. Layers housed in mobile cages on the pasture laid eggs of an average weight of 66.97 g, or 5.6% heavier than eggs from conventional cages (63.22 g). The shell weight of eggs from mobile cages was significantly higher (9.45 vs 8.73 g), which contributed to an increase in the share of egg shell in the structure of these eggs by 0.3% compared to conventional cage eggs. The ratio of albumen/yolk in eggs from mobile cages was lower compared to conventional cages, similar to the previous

comparison of floor and cage systems. The yolk colour was more intense with the access to pasture in mobile cages.

Table 2. Quality of eggs from conventional cage system and mobile cages on the range system at the age of layers of 51-53 weeks

Parameter	Conventional cages		Mobile cages on the pasture		p
	Mean	SD	Mean	SD	
Egg weight, g	63.22	4.38	66.97	5.31	0.0194
Egg shape index	76.20	2.02	77.35	2.58	0.125
Shell colour, points	3.65	0.59	3.70	0.57	0.786
Shell cleanness, points	4.90	0.31	4.70	0.73	0.267
Shell deformation, mm	0.209	0.032	0.221	0.041	0.333
Shell weight, g	8.73	0.76	9.45	0.77	0.0053
Shell thickness, mm	0.352	0.022	0.337	0.034	0.105
Shell breaking force, kg	2.37	0.53	2.40	0.41	0.843
Albumen height, mm	7.455	1.234	8.205	1.504	0.0929
Albumen/Yolk ratio	2.11	0.23	2.02	0.22	<0.0001
Yolk colour, Roche	11.77	0.63	13.25	0.64	<0.0001
Haugh Units	84.60	7.02	87.20	9.66	0.336

In agreement with the obtained results suggesting that free range housing leads to an increase in the weight of eggs, are results presented by *Rossi et al. (2007)*. *Holt et al. (2011)* present similar results and bring them in conjunction with different ambient conditions in the conventional cage and free range system, primarily temperature. *Minelli et al. (2007)* emphasize the importance of nutrition and conclude that the housing system itself has no decisive importance on the weight of the eggs. In this regard, the results of our research can be discussed, as the layers in mobile cages had available pasture area within the cages, as an additional source of nutrition and more favourable environmental conditions compared to the conventional cage system. The lower average weight of eggs in the free range system *Krawczyk (2009)* explains the result of the conditions of nutrition, when the protein requirements of layers on the range are not met. A significantly higher share of egg shell in the structure of eggs from the free range system, stated by *Lewko and Gornowicz (2011)*, is confirmed by our results. One of the parameters of the quality of eggs from free range that meets consumer expectations is the yolk colour (*Svobodova et al., 2014; Sokolowicz et al., 2018*). Stronger pigmentation of egg yolk from the mobile cages is the result of additional intake of natural pigments from pasture, in addition to the equal content of synthetic pigments in the feed mixture of both groups of layers.

Conclusion

In conditions of the same genetic basis and age of the layers, the parameters of the eggs quality that are significantly affected by the housing system are the egg weight, certain properties of eggshell quality (cleanliness, colour and weight), the albumen/yolk ratio and the yolk colour. The housing system in the mobile cages on the pasture, as well as the floor pen system, showed an advantage over conventional cages for most of the mentioned parameters except for the cleanliness of the shell. From the aspect of creating the conditions for more intensive development of alternative table egg production systems, the presented results indicate the possibility of improving certain quality properties, important for producers, but also those that satisfy consumer preferences.

Kvalitet jaja komercijalnih hibridnih nosilja u različitim sistemima gajenja

Zdenka Škrbić, Miloš Lukić, Veselin Petričević, Snežana Bogosavljević-Bošković, Simeon Rakonjac, Vladimir Dasković, Nataša Tolimir

Rezime

Parametri kvaliteta jaja hibridnih nosilja su analizirani uporednim ispitivanjem konvencionalnog kaveznog i alternativnih sistema gajenja: podnog (u boksevima) i u mobilnim kavezima na pašnjaku. Analiza kvaliteta jaja iz konvencionalnog kaveznog i podnog sistema gajenja obavljena je u uzrastu nosilja 42 do 44 nedelje a iz konvencionalnog kaveznog i mobilnih kaveza na pašnjaku, u periodu 51 do 53 nedelje starosti nosilja. Svaki ogled je organizovan u 3 ponavljanja u 3 sedmodnevna perioda i analizirano je 30 jaja po ponavljanju, odnosno 90 po sistemu gajenja. Ukupno je analiziran kvalitet 360 jaja. Ispitivan je inicijalni kvalitet jaja baziran na osobinama spoljašnjeg i unutrašnjeg kvaliteta jaja i kvaliteta ljuske. U uslovima iste genetske osnove i uzrasta nosilja, parametri kvaliteta jaja koji su bili pod značajnim uticajem sistema gajenja su težina jajeta, pojedine osobine kvaliteta ljuske (čistoća, boja i težina), odnos belanca i žumanca, kao i boja žumanca. Signifikantno veću težinu jaja i manji odnos belanca i žumanca su imala jaja iz alternativnih sistema gajenja u odnosu na kavezni sistem. Jaja iz podnog sistema su imala tamniju i prljaviju ljusku u odnosu na

konvencionalni kavezni sistem. Težina ljuske i boja žumanca su bili signifikantno veći kod jaja iz mobilnih kaveza na pašnjaku.

Ključne reči: nosilje, kvalitet jaja, sistem gajenja

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PREFERENCES OF CONSUMERS' FROM SERBIA TOWARD ORGANIC EGGS

**Tolimir Nataša¹, Maslovarić Marijana¹, Škrbić Zdenka², Rajković Borislav³,
Radišić Robert¹, Lukić Miloš²**

¹Institute for Science Application in Agriculture, Bulevar Despota Stefana 68b, 11000, Belgrade, Republic of Serbia

²Institut for Animal Husbandry, Autoput 16, 11080 Belgrade-Zemun, Republic of Serbia

³Faculty of Agriculture, Nemanjina 6, 11080, Belgrade-Zemun, Republic of Serbia

Corresponding author: Nataša Tolimir, ntolimir@ipn.co.rs

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Abstract: In order to examine consumers' preferences toward eggs from organic production and their willingness to allocate more money for these eggs, 581 respondents were surveyed in three regions of Serbia (the Belgrade region, the Šumadija and the Western Serbia region, and the Southern and Eastern Serbia region). Through the survey questionnaire, respondents declared themselves whether they are buying organic eggs and how much money they are ready to allocate for this type of eggs. Answers from surveyed respondents who are buying organic eggs were analyzed by gender, age, status, education, monthly income and the region in which they are living. According to the survey results, it can be concluded that in Serbia, there is a very small number of consumers who are buying organic eggs (2.41%), and that all of them are located in the Belgrade region. Furthermore, only 8.34% of consumers were willing to pay 30% higher price for organic eggs, on average for all three regions. Analyzing data within the category of organic eggs consumers, the highest tendency for buying organic eggs was determined for female respondents (76.92%), those with the higher education level (61.54%), the employed respondents (92.31%), the respondents aged from 36 to 55 (61.52%) and the respondents with the highest monthly household income (46.15%). The results of the present study indicate that the percentage of consumers who buy organic eggs in Serbia is very small, it is present only in certain regions and the willingness to pay more for organic eggs is low.

Key words: organic eggs, consumer, survey, regions, Republic of Serbia

Introduction

Statistical data on the organic eggs production in Serbia do not exist, but in the database of organic products producers led by the Ministry of Agriculture, Forestry and Water Management of the Republic of Serbia, on the basis of the reports from authorized control organizations for performing the control and certification of this production (*Directorate for National Reference Laboratories, 2019*), the number of laying hens in the status of organic production is monitored and recorded. According to the data from this database, the number of laying hens increased from 1,079 (2015) to 3,122 (2016), or 4,415 (2017). According to the same data source, observed regionally, in 2017 the largest number of 1079 (2,595) was found in the region of Šumadija and Western Serbia, and in the region of Vojvodina (1,515), while a significantly smaller number was recorded in Belgrade (200) and Southern and Eastern Serbia region (105). When viewed in relation to the total production of 1,759 million eggs in 2017 in Serbia (*Statistical Yearbook of the Republic of Serbia, 2018*) the production of organic eggs is modest, regardless the trend of increasing the number of hens in this production.

In Europe, organic poultry production, in the period from 2007 to 2015, recorded the highest increase (by 108%) within the organic livestock production (*Willer et al., 2017*). The statistical data indicate a tendency of increasing the number of poultry from "non-cage" systems (*Rakonjac et al., 2017*), which can be related to the major changes in the legal regulations in the field of animal welfare, sustainable and ecological production, food safety, as well as with increasingly pronounced attitudes and market demands of the customers. According to the *European Commission (2018)* data, the largest share of laying hens in the organic production system is in Denmark (31.1%), Sweden (16.1%), Germany (12%), Austria (11.6%) and Luxembourg (10.5 %).

Market monitoring in Europe in 2017 (*Willer et al., 2019*) has shown that the largest share of organic eggs in the total market value of organic products was in Denmark (32.6%), France (29.6%), Switzerland (26.6 %), Austria (21.6%) and Germany (21%), followed by Finland, the Netherlands, Belgium and Norway (15 to 20%). In Serbia there is no official data on the market share of organic eggs, and according to the research by *Tolimir et al. (2017)* conducted on the Belgrade market, as the largest in the country, organic egg supply is characterized by a small number of producers/suppliers, with sales only in some hyper/super-markets, a relatively small number of specialized organic food stores and several markets with special section for organic products.

According to the research conducted by *Rodić et al. (2010)*, in Serbia 26% of table eggs consumers would choose organic eggs, in the case they have the opportunity to choose. On the other hand, according to the research by *Tolimir et*

al. (2017), only 4% of table egg consumers in the City of Belgrade decide to buy organic eggs. According to research by *Vehapi (2015)*, the most significant consumer's motivation for purchasing organic food is health. Also, *Perić et al. (2015)* indicated that table egg consumers in Serbia choose organic eggs because they consider these eggs to be better for their health compared to eggs from conventional production. Among the most important reasons restricting or preventing the purchase of organic products *Vehapi (2015)* indicates the price, and *Vlahović and Šojić (2016)* point out that, besides the price, a significant limiting factor is also low purchasing power, which is a consequence of the low income. The aim of this paper is to determine the consumers' preferences in Serbia toward organic eggs, as compared to eggs from other production systems, as well as consumers' willingness to allocate more money for organic eggs compared to those from conventional production. At the same time, the purpose of the paper is to identify preferences in the group of organic egg consumers depending on the gender, education, status, age and the amount of income.

Material and Methods

Survey questionnaire included 581 consumers' on the territory of the Republic of Serbia, from three regions: Belgrade region (291 respondents), Šumadija and Western Serbia (158 respondents), and Southern and Eastern Serbia (132 respondents) region, out of a total of five, according to the regional division of Serbia (*Statistical Office of the Republic Of Serbia, 2018*).

The structured survey questionnaire consisted of:

a) information about the respondents obtained by completing the offered answers for the following categories: gender (male, female), education (secondary school, higher education), status (student, employed, retired), place of residence (entering), age (less than 18, 26-35, 36-55, 56-75) and the amount of income (30,000-40,000 RSD, 40,000-70,000 RSD and more than 70,000 RSD).

b) questions with the offered answers: 1) 'Which eggs do you usually buy?' - offered answers: eggs from conventional production, eggs from 'traditional' production, organic eggs and eggs from functional food programs (ω -3 fatty acids or selenium enriched omega eggs) etc.; 2) 'How much are you willing to pay more for eggs from organic production?' - offered answers: I am not ready to pay more, I am willing to pay less than 30% and I am willing to pay more than 30%.

For the group of respondents which stated to purchase organic eggs, the structure of customers was established by computing the share of consumers

according to the categories, more precisely putting in relation number of buyers according to the individual category (gender, education level, age, income level) with the total number of consumers within this group.

When processing the data, only questionnaires for which the examinees marked the category and answered the question were included in the analysis. Standard methods of analysis in Microsoft Excel program were used.

Results and Discussion

The results related to the consumers' preferences regarding the choice of eggs from different production systems/technologies in different regions of the Republic of Serbia are shown in the Table 1.

Table 1. Consumers' habits when buying table eggs in Serbia

Answers to the question: "Which eggs do you usually buy?"					
Eggs from different production systems	Branded eggs from conventional production	Eggs from traditional production (green market)	Organic eggs	Eggs from the functional food program	Other
Region					
Belgrade (%)	29.90	54.98	4.12	0.00	11.00
Šumadija and Western Serbia (%)	10.13	84.17	0.00	0.00	5.70
Southern and Eastern Serbia (%)	10.61	81.82	0.00	0.00	7.58
Total (%)	20.14	68.67	2.41	0.00	8.78

By examining the most common consumer preferences in Serbia toward eggs from different production systems/technologies (Table 1), differences depending on the region have been found. In total, the majority of consumers/customers tend to buy eggs from traditional systems at the green market, but it was found that in Belgrade region, only 54.98% of consumers buy eggs from traditional systems (at the green market), while this percentage is much higher the region of Šumadija and Western Serbia - 84.17%. In Belgrade region, a significant share of the consumers choose eggs of certain brand in hyper/supermarkets (about 30%), compared to the consumers in the Šumadija and Western Serbia (10.13%) and Southern and Eastern Serbia (10.61%) regions. The share of consumers who decide to buy organic eggs is very low, 2.41% on average in central Serbia, whereby, viewed by regions, it is 4.12% in the region of Belgrade,

while there are no consumers of this type of table eggs in the remaining two regions.

Compared to Serbia where there is an extremely small share of organic egg consumers/customers, in Poland consumers are having extremely positive opinion about organic food, and 65% of them stated that they buy organic eggs (*Žakowska – Biemans and Tekien, 2017*). One of the reasons for the low consumers' commitment in Serbia for the purchase of organic eggs may be a limited market offer of organic eggs, to which more attention should be paid in the coming period, especially in Šumadija and Western Serbia region, and the Southern and Eastern Serbia region. Researches world-wide suggest that market chains are considered to be factors that initiate the organic product market (*Rader et al., 2018*). Organic eggs have a significant place throughout European countries with a developed organic market with a share over 20% in Denmark, France, Switzerland, Austria and Germany (*Willer et al., 2019*).

The willingness of egg consumers in Serbia, according to the region, to pay more money for organic eggs is presented in the Table 2.

Table 2. Willingness of egg consumers to pay a higher price for organic eggs

Answers to the question: "How much are you willing to pay more for eggs from organic production?"			
Answers			
Region	I am not willing to pay more %	I am willing to pay more	
		Less than 30%	More than 30%
Belgrade region	28.82	58.78	12.40
Šumadija and Western Serbia region	25.45	66.46	8.09
Southern and Eastern Serbia region	28.79	66.67	4.55

Share of consumers in Serbia who expressed their readiness to allocate more money assets for organic eggs compared to conventionally produced eggs ranged from 71.18 to 74.55%, but those who expressed their readiness to single out more than 30% for organic eggs were in small percentage, ranging from 4.55% in the Southern and Eastern Serbia region to 12.4% in the Belgrade region. Considering that *Tolimir et al. (2018)* established that the price of organic eggs is greater than 2.05 to 3.07 times compared to conventionally produced eggs, i.e. that organic eggs are more expensive than the conventional ones from 104.7 to 207.7%, one can understand the result of this study, according to which only 2.41% of consumers in Serbia are choosing the organic eggs. The higher price of organic eggs is a result of higher production costs, and in Germany the price of organic production is at least twice as large compared to conventional ones (*Schaack et al., 2011*).

Also, it can be stated that in Serbia, the highest percentage of consumers/customers express the willingness to allocate up to 30% more funds for organic eggs, depending on the region from 58.78% to 66.67%. According to the research findings of *Rodić et al. (2010)* consumers in Serbia have shown willingness to single out 20% more money for eggs from “no cage” production systems. It is important to note that some authors point out that the high price of organic food negatively affects consumers' attitudes, which might be considered to be conditionally a barrier when purchasing them (*Lee and Yun, 2015; Vehapi, 2015*), whereas a number of authors established consumers' readiness in the EU and in the USA to pay higher price for organic products within a range from 10 to 30% (*Vapa - Tankosić et al., 2017*).

The results of the questionnaire survey obtained through the analysis within the consumer/customer of organic food group indicate the differences between individual categories regarding their preferences (Table 3).

Among the customers of organic eggs, the proportion of female sex was higher in comparison to male, those with higher education compared to the secondary education, those employed compared to students and pensioners, those aged between 36 and 55 compared to members of the younger and older population, with higher level of income the share of consumers/customers grew.

Table 3. Structure of organic eggs consumers

Structure of organic eggs consumers in Belgrade region	
Characteristic	%
Gender	
Male	23.08
Female	76.92
Education	
Secondary school	38.46
Higher education	61.54
Status	
Student	0
Employee	92.31
Pensioner	7.69
Age	
18-25	0
26-35	0
36-55	61.52
55-75	38.46
Monthly income (RSD)	
30,000-40,000	15.38
40,000-70,000	38.46
>70,000	46.15

Šojić (2017) determined that purchasing of organic products in Serbia is influenced by the consumer's life age, education level and the level of income. Vapa-Tankosić *et al.* (2017) point out on the differences among consumers in the willingness to buy organic products, depending on the gender, level of education, age, place of residence, household size and income.

Conclusion

The results of the survey show that although there are some differences between the different regions in the Republic of Serbia, only very small share (2.38%) of consumers/customers in Central Serbia decide to buy eggs produced in the organic production system. The reasons may lie in the relatively limited supply of the high-quality organic eggs on the market on the one hand, and on the other hand in the readiness of only a small number of consumers/customers (4.5 to 12% depending on the region) to allocate more than 30% more money for the organic eggs than for the conventionally produced eggs. The analysis of consumers/customers of organic egg suggests that when it comes to the purchase of organic eggs, women commit to it three times more often compared to men, and that the largest number of consumers can be found in the category of employees, with high education and with the highest incomes, which can be perceived as a target group. The increase in consumers' preferences towards organic eggs would contribute to higher informational and educational level amongst the consumers, as well as the creation of conditions for the organic eggs to become more available to the organic eggs consumers/customers in Serbia. Examination of consumers' attitude to organically produced eggs in Serbia is scarce and in this regard, this paper serves the purpose of establishing the starting point for the development of organic egg market, more broadly the development of organic egg production.

Preference potrošača/kupaca u Srbiji prema jajima iz organske proizvodnje

Tolimir Nataša, Maslovarić Marijana, Škrbić Zdenka, Rajković Borislav, Radišić Robert, Lukić Miloš

Rezime

U cilju ispitivanja preferenci potrošača u Srbiji o jajima iz organske proizvodnje, kao i njihove spremnosti da izdvoje više novčanih sredstava za kupovinu jaja iz ovog sistema proizvodnje anketirano je 581 potrošač u tri regiona (beogradskom regionu, regionu Šumadije i Zapadne Srbije i regionu Južne i Istočne

Srbije). Kroz anketno ispitivanje potrošači su se izjasnili da li kupuju organska jaja i kolika je njihova spremnost da za jaja iz organskog sistema izdvoje više sredstava. Odgovori anketiranih potrošača koji kupuju organska jaja obrađeni su po polu, starosti, statusu, obrazovanju i sredini u kojoj žive. Prema rezultatima anketnog ispitivanja može se konstatovati da u Srbiji među potrošačima jaja je veoma mali udeo onih koji kupuju organska jaja (2,41%), kao i da su svi potrošači/kupci regionalno posmatrano iz jednog, odnosno Beogradskog regiona, dok ih nema u preostala dva ispitivanjem obuhvaćena regiona. Spremnost da plate više od 30% za jaja iz organske proizvodnje iskazalo je svega 8,34% potrošača, prosečno za sva tri regiona. Analizom podataka unutar kategorije potrošača organskih jaja najveća opredeljenost za kupovinu organskih jaja konstatovana je kod potrošača ženskog pola (76,92%), sa visokim obrazovanjem (61,54%), kod zaposlenih (92,31%), potrošača starosne dobi od 36 do 55 godina (61,52%) i sa najvećim mesečnim prihodom domaćinstva (46,15%). Rezultati rada upućuju na zaključak da se veoma mali udeo potrošača konzumnih jaja i to samo u pojedinim regionima Srbije opredeljuje za kupovinu organskih jaja, a da je spremnost da plate više za ova jaja niska. Povećanju preferenci potrošača prema organskim jajima doprinela bi bolja informisanost i edukovanje potrošača, kao i stvaranje uslova da organska jaja u pogledu cene i tržišne ponude budu dostupnija potrošačima/kupcima jaja u Srbiji.

Ključne reči: organska jaja, potrošač/kupac, anketa, region, Republika Srbija

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FATTY ACID COMPOSITION OF MUSCLE AND BACKFAT IN PIG BREEDS AND CROSSBREEDS

Teodora Popova¹, Jivko Nakev²

¹Institute of Animal Science - Kostinbrod, Bulgaria

²Agricultural Institute – Shumen, Bulgaria

Corresponding author: Teodora Popova, tpopova@yahoo.com

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Abstract: The aim of this study was to compare the fatty acid profile of *M. Longissimus thoracis* (*M. LT*) and the backfat of pigs from three breeds (Landrace, Pietrain, Duroc) and their crosses (Pietrain x Landrace and Duroc x Landrace). The fatty acid composition was determined through gas chromatography and the differences in the examined traits were statistically evaluated by one way ANOVA and Tukey post-hoc comparisons. The fatty acid profile of the intramuscular lipids revealed significant differences among the breeds and their crosses. Duroc pigs exhibited the highest percentage of the C14:0, however this breed and Duroc x Landrace crossbreed displayed lower content of polyunsaturated (PUFA) and monounsaturated fatty acids (MUFA) when compared to the rest. Higher content of PUFA was observed in Landrace, Pietrain and their cross. Furthermore, Pietrain pigs displayed the lowest content of C18:0, C18:1n-9 and MUFA but highest of C20:4n-6. Differences between breeds and crossbreeds in the fatty acid profile of the backfat were found only in the inner backfat layer concerning the content of C18:0, C18:1n-9, C20:4 n-6 and MUFA. C18:0 and C18:1n-9 displayed lower content in Pietrain gilts when compared to Duroc x Landrace and Duroc respectively. The total amount of MUFA was also significantly different between Pietrain and Duroc, with lower content in Pietrain. This breed exhibited higher content of C20:4n-6 in comparison to Landrace and both crossbreeds.

Keywords: Fatty acids, Muscle, Backfat, Pigs

Introduction

Fatty acid composition of meat is of great importance because of its effects on meat quality characteristics and its implication for human health. There has been an increased interest in recent years in the ways to manipulate the fatty acid composition of meat (Wood *et al.*, 2003). Meat is considered to be a major source of fat in the human diet and especially of the saturated fatty acids, implicated in diseases including various cancers and especially coronary heart disease.

The nutritional value of pork is determined to a great extent by the fat content in the carcass and fatty acids, especially those having hypocholesterolaemic effects such as C14:0 and C16:0. Similar to most of the production traits, the fatty acid composition of pork the adipose tissue, mainly backfat, is affected by various factors including fatness and body weight (*Raj et al., 2006*), dietary fatty acid intake (*Realini et al., 2010; Waszkiewicz-Robak et al., 2015; Raj et al., 2017*). There are also intrinsic factors such as sex (*Zhang et al., 2009*) and genetic background (*Parunović et al., 2013*). According to *Kasprzyk (2007)*, the fat deposition and its fatty acid composition are highly heritable and vary among and within breeds.

The aim of the study was to compare the fatty acid profile of the breeds and their crosses in both muscle and backfat.

Material and Methods

The study was carried out in the pig farm Golyamo Vranovo Invest AD with a total of 30 gilts divided into 5 groups as follows: Landrace (n=6), Pietrain (n=6), Duroc (n=6), Duroc x Landrace (n=6) and Pietrain x Landrace (n=6). During the finishing period the animals were reared according to the instructions described in the Regulation 21/14.12.2005 concerning the minimum requirements for protection and welfare for pig breeding when the animals are provided feed and water *ad libitum*. Two phase feeding was applied, as the first phase was in the period 40 - 80 kg, while the second phase was from 80 kg until slaughter. The diet composition is presented in Table 1.

After slaughtering, the pigs were skinned. The carcass weight was as follows: Landrace 83.39 kg (SEM 0.55), Pietrain 84.06 kg (SEM 0.89), Duroc 84.06 kg (SEM 0.89), Large White 84.84 kg (SEM 0.76), Duroc x Landrace 71.04 kg (SEM 0.32) and Pietrain x Landrace 70.56 (SEM 0.43). Samples for analysis of the fatty acid composition were taken from *M. LT* at the last rib of the left side of each carcass, as well as the inner and outer backfat layer at the same location. Total lipids from the tissues were extracted according to the method of Bligh and Dyer (1959). Methyl esters of the total lipids, isolated by preparative thin layer chromatography, were obtained using 0.01 % solution of sulfuric acid in dry methanol for 14 h, as described by Christie (1973). The fatty acid composition of the total lipids was determined by gas-liquid chromatography (GLC) analysis using a chromatograph C Si 200 equipped with a capillary column (DM-2330:30 m×0.25 mm×0.20 µm) and hydrogen as a carrier gas. The oven temperature was first set to 160°C for 0.2 min, then raised until 220°C at a rate of 5°C/min and then held for 5 min. The temperatures of the detector and injector were 230°C. Methyl esters were identified through comparison to the retention times of the standards.

Fatty acids are presented as percentages of the total amount of the methyl esters (FAME) identified (Christie, 1973).

Table 1. Diet composition

Components	Finisher I phase	Finisher II phase
Corn, %	21.00	18.00
Barley, %	23.30	23.40
Wheat, %	19.00	23.00
Soy meal, %	15.00	16.00
Sunflower expeller, %	18.00	16.00
Soy oil, %	2.40	2.40
Limestone, %	0.50	0.50
Panto Mix 3148 finisher, %	0.20	0.20
Hostazim + Optiphos, %	0.10	0.10
Lysine, %	0.20	0.20
Salt, %	0.30	0.20
Total	100.00	100.00
ME, kcal/kg	3424	3432
Crude protein, %	19.05	18.89
Crude fibers, %	5.37	5.14
Fat, %	5.93	5.77
Lysine, %	1.07	1.08
Methyonine, %	0.34	0.33
Methyonine + Cysteine, %	0.65	0.64
Trptophane, g	0.31	0.30
Ca, %	0.48	0.48
P, %	0.60	0.59

The data were statistically evaluated through one way ANOVA and Tukey post hoc comparisons. Pearson's Correlation Coefficient was used for the correlation analyses. The statistical procedures were performed using the JMP v. 7 software package.

Results and Discussion

As presented in Table 2, significant differences between the breeds and crosses were found in regard to the most of the individual fatty acids in *M. LT*. C14:0 displayed the highest content in the Duroc gilts, while the amount of C18:0 was the highest in the Duroc x Landrace animals.

Table 2. Fatty acid composition of *M. Longissimus thoracis* in the breeds and crosses

Fatty acids	Landrace	Pietrain	Duroc	Duroc x Landrace	Pietrain x Landrace	SEM	Sig.
C14:0	1.57a	1.54a	2.04b	1.53a	1.47a	0.06	**
C16:0	24.12	24.75	25.03	24.88	25.08	0.33	ns
C16:1n-7	3.03ab	3.01ab	3.40a	2.72b	2.47b	0.08	**
C18:0	12.71b	11.90c	11.84c	13.90a	12.98b	0.15	***
C18:1n-9	34.27b	31.93b	40.22a	37.16a	31.93b	0.82	***
C18:2n-6	18.01ab	19.40a	13.03b	15.23ab	19.66a	0.68	**
C18:3n-3	0.85	0.96	0.86	0.92	0.91	0.03	ns
C20:2n-6	0.45	0.54	0.49	0.48	0.54	0.02	ns
C20:3n-6	0.44ab	0.47a	0.30bc	0.28c	0.47a	0.02	***
C20:4n-6	3.79ab	4.52a	2.31c	2.48bc	3.93a	0.21	***
C20:5n-3	0.16	0.18	0.09	0.06	0.10	0.01	0.06
C22:5n-3	0.52ab	0.71a	0.34b	0.33b	0.46b	0.03	***
C22:6n-3	0.08	0.09	0.05	0.03	0	0.01	ns
SFA	38.40	38.19	38.91	40.31	39.53	0.40	ns
MUFA	37.30b	34.94b	43.62a	39.88ab	34.40b	0.86	***
PUFA	24.30ab	26.87a	17.47c	19.81bc	26.07ab	0.96	***

Sig. **P<0.01; ***P<0.001, ns- non significant. Means connected with different letters are significantly different (P<0.05)

The content of the major saturated fatty acid - C16:0 did not differ among groups. The content of C16:1n-7 was lower in the crossbred pigs but in Duroc gilts it reached the highest content of 3.40%. The levels of C18:1n-9 were higher in Duroc and Duroc x Landrace when compared to the other groups. On the other hand, these two groups exhibited lower levels of C18:2, C20:3n-6 and C20:4n-6. The highest amounts of these fatty acids were observed in Pietrain, Pietrain x Landrace and Landrace pigs. Furthermore, when compared to the rest of the groups, Pietrain pigs had higher amount of C22:5n-3. The total amount of the monounsaturated fatty acids was higher in Duroc gilts, however this groups had lower content of the total polyunsaturated fatty acids Together with Duroc x Landrace. Our results clearly indicated the considerable difference between the Duroc and the other breeds and crosses in the study. Similar to us, *Raj et al. (2010)* found that Duroc pigs had higher content of MUFA but lower in PUFA when compared to Belgian Landrace, Hampshire and Pietrain. However they found that Duroc had significantly higher SFA which in our study was observed only in regard to C14:0. *Choi et al. (2016)* also determined the highest content of C14:0 in Durocs in comparison to Landrace, Yorkshire and crossbred pigs, however in their study the pigs of Duroc breed exhibited the lowest MUFA which contradicts to what we observed. On the other hand, the crossbreed involving Duroc also displayed considerably lower total amount of PUFA and higher of MUFA. Similar to us, *Alonso et al.*, in their studies from 2009 and 2015 concluded that the

inclusion of Duroc breed in the crossbreeds led to significantly higher lower content of PUFA. However, they determined higher content of SFA in the crossbreeds involving Duroc, which we failed to observe. The higher amount of PUFA in Pietrain, Landrace and their cross that we found in our study was in line with the results of *Raj et al. (2005)* in Pietrain and Belgian Landrace. This could be explain with the lower intramuscular lipid content. In a previous study we significant negative correlation existing between these two traits (*Popova et al., 2015*). Also, as stated by *Raj et al. (2005)* the leaner pigs have greater response to dietary PUFA. In these pigs the *de novo* synthesis of SFA is less intensive when compared to the fatter pigs which is associated to differences in the PUFA deposition in the adipose tissue.

Contrary to the lipid profile of the muscle tissue, the differences between the breeds and the crosses that were observed in the backfat were considerably less. In the inner layer we found discrepancies in the content of C18:0, C18:1n-9, C20:4n-6 and the total amount of MUFA (Table 3).

Table 3. Fatty acid composition of the inner backfat layer in the breeds and crosses

Fatty acids	Landrace	Pietrain	Duroc	Duroc x Landrace	Pietrain x Landrace	SEM	Sig.
C14:0	1.92	1.91	1.82	1.75	1.80	0.04	ns
C16:0	23.76	24.00	23.77	24.46	24.34	0.39	ns
C16:1n-7	1.93	1.72	1.81	1.75	1.88	0.06	ns
C18:0	11.72ab	10.49b	11.84ab	12.39a	11.41ab	0.19	*
C18:1n-9	35.47ab	32.00b	37.14a	35.24ab	36.22ab	0.56	*
C18:2n-6	21.80	25.92	20.33	21.31	21.05	0.72	ns
C18:3n-3	1.99	2.20	1.75	1.95	1.89	0.06	ns
C20:2n-6	0.84	1.06	0.93	0.80	0.86	0.03	ns
C20:4n-6	0.57b	0.70a	0.65ab	0.35b	0.55b	0.02	*
SFA	37.40	36.40	37.39	38.60	37.55	0.54	ns
MUFA	37.40ab	33.72b	38.95a	36.99ab	38.10ab	0.59	*
PUFA	25.20	29.88	23.66	24.41	24.35	0.82	ns

Sig. *P<0.05, ns- non significant. Means connected with different letters are significantly different (P<0.05)

Both C18:0 and C18:1n-9 had lower content in Pietrain gilts in comparison to the Duroc x Landrace and Duroc respectively. The total amount of MUFA was also significantly different between Pietrain and Duroc, displaying lower content in Pietrain. On the other hand this breed exhibited higher content of C20:4n-6 in comparison to Landrace and both crossbreeds. Our results are partly in line with *Raj et al. (2010)* in regard to the percentage of C18:0, C18:1n-9 and MUFA, however, these authors did not find any significant difference in the percentage of C20:4n-6. The fatty acid composition of the outer backfat layer did not differ

between the groups, except with the tendency toward higher percentage of C18:0 in the Duroc and crossbred pigs (Table 4).

Table 4. Fatty acid composition of the outer backfat layer in the breeds and crosses

Fatty acids	Landrace	Pietrain	Duroc	Duroc x Landrace	Pietrain x Landrace	SEM	Sig.
C14:0	1.76	1.59	1.73	1.66	1.71	0.05	ns
C16:0	22.87	22.32	23.06	23.55	22.95	0.33	ns
C16:1n-7	2.14	1.78	1.85	1.92	1.95	0.06	ns
C18:0	9.93	9.92	10.60	11.35	10.42	0.18	ns
C18:1n-9	35.47	36.16	36.19	35.95	38.34	0.47	ns
C18:2n-6	24.08	24.45	22.96	22.14	21.26	0.57	ns
C18:3n-3	2.28	2.14	2.02	2.02	1.93	0.06	ns
C20:2n-6	0.85	1.00	0.93	0.82	0.88	0.03	ns
C20:4n-6	0.62	0.64	0.66	0.59	0.56	0.01	ns
SFA	34.56	33.83	35.39	36.56	35.08	0.45	ns
MUFA	37.61	37.94	38.04	37.87	40.29	0.46	ns
PUFA	27.83	28.23	26.57	25.57	24.63	0.66	ns

ns- non significant

Regardless of the breed, as presented in Table 5, significant differences in some of the selected fatty acids existed between the tissues.

Table 5. Difference between some selected fatty acids in the muscle and backfat

Fatty acids	<i>M. LT</i>	Inner layer	Outer layer	SEM	Sig.
C14:0	1.63a	1.84b	1.69ab	0.03	*
C16:0	24.77a	24.07ab	22.95b	0.22	**
SFA	39.07a	37.48b	35.08c	0.32	***
C18:1n-9	35.10	35.21	36.42	0.38	ns
MUFA	38.03	37.03	38.35	0.39	ns
C18:2n-6	17.07b	22.08a	22.97a	0.48	***
C18:3n-3	0.90a	1.96b	2.08b	0.06	***
C20:4n-6	3.41a	0.56b	0.61b	0.16	***
PUFA	22.90a	25.49ab	26.57b	0.51	**

Sig. *P<0.05; **P<0.01; ***P<0.001, ns- non significant. Means connected with different letters are significantly different (P<0.05)

Among all three, the muscle had the highest content of SFA, while the outer backfat layer had the lowest. This difference was due to the highest amount of C16:0 in the muscle. On the other hand the content of C14:0 was lower in the muscle when compared to the inner and outer backfat layer. The difference between the two layers in regard to C14:0 and C16:0 followed the same pattern, as the outer layer displayed lower percentage of these two fatty acids. The total

amount of MUFA and its major component C18:1n-9 did not differ between the tissues. The content of PUFA showed significant differences in both total and some of the individual fatty acids. The total content of PUFA was lower in the muscle tissue when compared to the backfat and especially with the outer layer. Both C18:2n-6 and C18:3n-3 displayed considerably higher percentage in the both backfat layers, however the content of C20:4n-6 was much higher in the muscle. In a previous study comparing the fatty acid composition of the *M. Longissimus dorsi* and the layers of the backfat (Popova et al., 2015) we observed higher content of SFA in the intramuscular lipids due to higher percentage of C16:0 higher content of the PUFA coinciding with the results of the present study. In line with our results, Raj et al. (2010), Dominguez and Lorenzo (2014) also found higher content of C18:2n-6 and C18:3n-3 in the backfat when compared to the intramuscular fat of *M. Longissimus dorsi*, but lower C20:4n-6. Such differences were observed in other species (He et al., 2005). It could be suggested that the adipose and muscle tissue differ in their uptake, use and storage of these fatty acids. C18:2n-6 and C18:3n-3 in pigs are essential and derived from the diet. According to Nguyen et al. (2003), the intake of linoleic acid is much higher than required for membrane synthesis, and a high proportion of this acid is channelled into a storage site, such as the adipose tissue. However, α -linolenic acid is probably more efficiently incorporated into intramuscular fat than into adipose tissue. According to He et al. (2005) the higher content of C20:4 in the intramuscular fat might be due to the higher concentration of phospholipids in the membrane of the muscles, that are richer in long chain PUFA than tryacylglycerols.

Table 6 showed that significant negative correlations were found between the total SFA and PUFA ($P < 0.05$), and also between the MUFA and PUFA ($P < 0.001$) in the muscle tissue, as the coefficient determined between SFA and PUFA was moderate.

Table 6. Correlations between the main classes of fatty acids in the muscle and backfat layers

<i>M. LT</i>	SFA	MUFA	PUFA
SFA	1.000	0.020	-0.437*
MUFA		1.000	-0.907***
PUFA			1.000
Innner layer			
SFA	1.000	0.066	-0.700***
MUFA		1.000	-0.759***
PUFA			1.000
Outer layer			
SFA	1.000	0.020	-0.699***
MUFA		1.000	-0.723***
PUFA			1.000

Sig. * $P < 0.05$; *** $P < 0.001$

The same correlations were found in the two backfat layers. The correlations determined confirm the results presented in Tables 2, 3 and 4 where the breeds and crossbreeds with highest content of MUFA displayed lowest amount of PUFA.

Conclusions

To conclude, the analysis of the fatty acid profile of the intramuscular lipids revealed considerable differences among the breeds and their crosses. The Duroc breed exhibited highest content of the C14:0, however this breed and the crossbreed that involved it displayed lower content of PUFA and MUFA in comparison to the rest. Higher content of PUFA was exhibited by Landrace, Pietrain and their cross. Pietrain breed however displayed the lowest content of C18:0, C18:1n-9 and MUFA but highest of C20:4n-6 among all. Differences between breeds and crossbreeds in the fatty acid profile of the backfat were found in regard to the inner backfat layer, concerning the content of C18:0, C18:1n-9, C20:4n-6 and the total amount of MUFA. Both C18:0 and C18:1n-9 displayed lower content in Pietrain gilts in comparison to Duroc x Landrace and Duroc respectively, while the total MUFA was also significantly different between Pietrain and Duroc, with lower content in Pietrain. The latter exhibited higher content of C20:4n-6 in comparison to Landrace and both crossbreeds.

Profil masnih kiselina mišićnog i masnog tkiva kod svinja jedne rase i meleza

Teodora Popova, Jivko Nakev

Rezime

Cilj ove studije bio je upoređivanje profila masnih kiselina *M. Longissimus thoracis* (M. LT) i ledne slanine svinja tri rase (landras, pijetren i durok) i njihovih meleza (pijetren x landras and durok x landras). Profil masnih kiselina određen je gasnom hromatografijom, a razlike u ispitivanim osobinama su statistički procenjene korišćenjem One way ANOVA i Tukey post-hoc upoređivanjem. Profil masnih kiselina intramuskularnih lipida pokazao je značajne razlike između rasa i njihovih meleza. Durok svinje su imale najveći procenat C14: 0, međutim ova rasa i melezi durok x landras pokazali su niži sadržaj polinezasićenih (PUFA) i mononezasićenih masnih kiselina (MUFA) u odnosu na ostale. Viši sadržaj PUFA primećen je kod grla rasa landras, pijetren i njihovim melezima. Pored toga, svinje pijetren rase su pokazale najniži sadržaj C18:0, C18:1n-9 i MUFA, ali najviši C20:4n-6. Razlike između rasa i meleza u profilu masnih kiselina ledne masti

utvrđene su samo u unutrašnjem sloju masne kiseline koji se odnosi na sadržaj C18:0, C18:1n-9, C20:4 n-6 i MUFA. C18:0 i C18:1n-9 su pokazali niži sadržaj u pijetren nazimicama u poređenju sa durok x landras i durok, respeltivno. Ukupna količina MUFA se takođe značajno razlikovala između pijetrena i duroka, sa nižim sadržajem u pijetrenu. Ova rasa je pokazala veći sadržaj C20:4n-6 u poređenju sa landrasom i oba meleza.

Ključne reči: masne kiseline, mišić, ledna slanina, svinje

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INFLUENCE OF SIRE BREED ON MEATINESS OF PIG CARCASS

Vladimir Živković¹, Łukasz Migdał³, Władysław Migdał³, Čedomir Radović¹, Marija Gogić¹, Slavča Hristov², Nenad Stojiljković¹

¹Institute for Animal Husbandry, Belgrade-Zemun, Republic of Serbia

²Faculty of Agriculture, University of Belgrade, Belgrade-Zemun, Republic of Serbia

³Faculty of Animal Science, University of Agriculture in Kraków

Corresponding author: Vladimir Živković, vladimirzivkovic_87@yahoo.com

Original scientific paper

Abstract: The aim of this paper was to determine the influence of sire breed on meatiness of pig carcass. Trial was conducted on 480 pigs, offspring of six boars, of two genotype Large White (LW) and Swedish Landrace (SL). Average body mass of examined animals were 114.5 kg. Share of meat yield of pig carcass was estimated on the slaughter line using two methods. First method use “two point” system where first measurement is depth of *m. longissimus* measured as the shortest link/connection between front (cranial) end of *m. gluteus medius* and the top (dorsal) rim/edge of the spinal canal and second is where *m. gluteus medius* penetrates the fat tissue the most. Second method also use two measurements: X1=thickness of fat tissue (with skin) in millimeters measured on medial carcass surface between 3rd and 4th lumbal vertebrae, caudo-cranially and X2=thickness of fat tissue (with skin) in milimeters measured on medial carcass surface between 3rd and 4th rib, caudo-cranially. The meatiness of the progeny very significantly depended on boar genotype ($p<0.01$). Offspring of SL boars had better results in both methods (60.47; 56.45%), than LW offspring (60.35; 55.85%).

Key words: offspring, backfat, slaughter characteristics

Introduction

The quality of pig carcasses is major topic in pig production, which always deserves attention of science as well as in practice. Quality of pig carcasses is measured in high share of muscle tissue in major carcass side parts, especially the most important parts. Traits of the pig carcass quality vary under the influence of genetic and environmental factors (breed, sires, breeding methods, age, castration, nutrition, season, etc.). Genotype of sires affects the variability of carcass quality traits of offspring (Petrović *et al.*, 2004; Pušić and Petrović, 2004), as well as sires

within the same genotype (*Kosovac et al., 1998; Petrović et al., 2002; Radović et al., 2003*). Results of some studies show that there are differences between breeds in regard to the effect of sire on variability of traits of the offspring (*Mijatović et al., 2005*) or the sire effect is not significant in different herds (*Bahelka et al., 2004*). Sires within three breeds have affected the variation in the content of meat in the carcass of performance tested sons (*Mijatović et al., 2005*), but boars of two breeds had no effect on depth of *m. longissimus dorsi* (MLD) in their offspring. For the purpose of investigation of lean meat and quality of meat deriving from pigs of different genotypes, we analyzed all relevant indicators of lean meat using different methods of investigation. Objective of this paper was to determine the influence of sire breed on meatiness of pig carcass.

Materials and Methods

Trial was conducted on 480 pigs, offspring of six boars, of two genotype Large White (LW) and Swedish Landrace (SL). Average body mass of examined animals were 114.5 kg. On slaughter line the meat yield was evaluated in two ways:

First method use “two point” system where first measurement is depth of *m. longissimus* measured as the shortest link/connection between front (cranial) end of *m. gluteus medius* (S), and the top (dorsal) rim/edge of the spinal canal and second is where *m. gluteus medius* penetrates the fat tissue the most (M) (*Regulations on the quality of pig carcasses and halves on the slaughter line, National newspapers 13/06*).

$$M\% = 47,978 + (26,0429 \times S/M) + (4,5154 \times M) - (2,5018 \times \log S) - (8,4212 \times S)$$

Second method is also method of two points, where for the assessment of carcass side meat yield measures of fat tissue on two points were taken: X1= thickness of fat tissue (with skin) in milimeters measured on medial carcass surface between 3rd and 4th lumbal vertebrae, caudo-cranially, and X2 = thickness of fat tissue (with skin) in milimeters measured on medial carcass surface between 3rd and 4th rib, caudo-cranially. Linear measures X1 and X2 were taken manually using ruler and mathematical formula calculated through setting of linear multiple regression model with two independent variables, defined by *Džinić et al. (2004)* and meat yield of each pig carcass side was calculated.

$$Y = 67.098 - 0.505X1 - 0.14X2$$

The data obtained were processed using the software package "STATISTICA" (Stat Soft Inc, 2012). ANOVA was used while the Tukey test served to determine the statistical significance of the differences between individual means values.

Results and Discussion

The results were shown in Table 1. suggests that there are major significant differences between boars, and between methods. Boars A, B, C are genotype SL, and D, E, F are LW. Boars A, C, D, F had significantly larger carcass side mass than B and E boars. Second method shows less percentage of meatiness in carcass sides. Carcass quality of offsprings significantly depended on boar genotype. Offsprings of SL boars had better percentage of meatiness in both methods (60.47; 56.45%), than LW offspring (60.35; 55.85%). Offsprings of boar C has shown significantly better results in meatiness in both methods than the rest of the boars.

Table 1. The effect of genotype on meatiness of carcass

	Boars						SEM	p
	A	B	C	D	E	F		
MCCS, kg	48.999 ^a	47.169 ^b	47.836 ^a	48.846 ^a	46.731 ^b	47.956 ^a	0.242	p<0.01
S, mm	17.663 ^a	16.013 ^{ab}	12.413 ^c	17.250 ^a	16.188 ^{ab}	14.200 ^{bc}	0.232	p<0.01
M, mm	90.463 ^c	98.600 ^a	91.600 ^{bc}	98.288 ^a	95.925 ^a	93.713 ^b	0.384	p<0.01
I method, %	57.903 ^c	60.710 ^b	62.814 ^a	59.637 ^b	60.116 ^{bc}	61.292 ^b	0.183	p<0.01
X1, mm	23.725 ^{ac}	23.688 ^{ac}	22.450 ^{bc}	24.613 ^{abc}	26.275 ^a	24.875 ^a	0.229	p<0.01
X2, mm	17.738 ^a	13.688 ^{bcd}	12.450 ^{cd}	14.613 ^{bcd}	16.275 ^{ab}	14.875 ^{bc}	0.244	p<0.01
II method, %	54.819 ^d	56.870 ^{ab}	57.668 ^a	56.263 ^{abc}	55.201 ^{cd}	56.104 ^{bcd}	0.153	p<0.01

SEM, Standard error of the means; MCCS, Mass of cooled left carcass sides; ^{a, b, c, d} in a row, the least squares means with a different superscript differ significantly (p<0.01)

Kosovac et al. (2007) using *Džinić et al. (2004)* model in their research stated lower values of meat percentage in carcass sides (49.20 – 55.52%) compared to values obtained in this research with ultrasound device, which are in concordance with values obtained by *Tomović, (2002)*. However, our data on yield of muscle tissue in carcass sides obtained by method of “two points” are lower than results stated by *Kušec et al., (2006)* and *Zekić et al., (2007)*. *Kralik et al. (1996)* suggested that meatiness of the progeny significantly depended on boar genotype.

Conclusion

The overall results showed that sire breed has major effect on carcass quality. However, there is large difference between methods. Further studies must be conducted to clarify why there is so much difference in final results.

Uticaj rase oca na mesnatost polutki svinja

Vladimir Živković, Łukasz Migdal, Władysław Migdal, Čedomir Radović, Slavča Hristov, Marija Gogić, Nenad Stojilković

Rezime

Cilj ovog rada je bio da se utvrdi uticaj rase nerasta na mesnatost polutki. Ogled je izveden na 480 životinja, potomaka 6 nerastova, rasa Švedski Landras (ŠL) i Veliki Jorkšir (VJ). Prosečna masa ispitanika je iznosila 114,5 kg. Mesnatost polutki je procenjena na liniji klanja korišćenjem dve metode. Prvi metod koristi dve tačke kao osnovu za procenu. Prva mera je dubina *m. longissimus* izmerena na mestu gde je najkraća konekcija od kranijalnog dela *m. gluteus medius* do dorzalnog dela kičmenog kanala, dok je druga mera gde *m. gluteus medius* prodire u masno tkivo najviše. Druga metoda takođe koristi “dve tačke”: X1= debljina ledjne slanine (sa kožom) između trećeg i četvrtog lumbalnog pršljena, X2= debljina ledjne slanine (sa kožom) između trećeg i četvrtog rebra. Mesnatost je veoma značajno varirala između genotipova ($p<0,01$). Potomci nerastova rase ŠL su imale bolje rezultate mesnatosti kod obe metode (60,47; 56,45%), u odnosu na potomke rase VJ (60,35; 55,85%).

Ključne reči: potomci, ledna slanina, klanične karakteristike

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ELECTRICAL CONDUCTIVITY OF DIFFERENT TYPES OF THE SERBIAN HONEY

Milica Živkov Baloš, Sandra Jakšić, Nenad Popov, Suzana Vidaković, Dragana Ljubojević Pelić, Jasna Prodanov Radulović, Željko Mihaljev

Scientific Veterinary Institute “Novi Sad”, Rumenački put 20, Novi Sad
Corresponding author: Milica Živkov Baloš, milica@niv.ns.ac.rs
Original scientific paper

Abstract: In this paper, the electrical conductivity of various types of honey collected from the Republic of Serbia was investigated. Electrical conductivity of honey has an important role in defining the overall properties and assessing the quality of honey. In accordance with the regulation concerning the quality of honey in the Republic of Serbia, minimum electrical conductivity in honeydew put in the market is set to 0.8 mS/cm. For other types of honey, the maximum permitted value of electrical conductivity is 0.8 mS/cm. A total of 138 samples of honey of different botanical origin were examined. The values of electrical conductivity were in the range between 0.08 and 1.99 mS/cm. Out of the total number of samples, 11 samples did not meet the requirements of relevant Serbian Regulations on the quality of honey due to inadequate electrical conductivity. The data indicated the lowest conductivity in acacia and rapeseed honey samples, while the highest conductivity was found in samples of forest and linden honey. Electrical conductivity is a useful tool for discriminating honeys of different botanical origin. For the purpose of more reliable confirmation of the botanical origin of honey it is necessary to perform pollen analysis of honey.

Key words: honey, electrical conductivity

Introduction

Honey is defined as a “natural sweet substance produced by honey bees from the nectar of plants or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which the bees collect, transform by combining with specific substances of their own, deposit, dehydrate, store, and leave in the honey comb to ripen and mature” (*Codex Alimentarius*, 2001). The major components of honey are sugars, although other minor components, such as enzymes, proteins, organic acids, minerals, pollen grains, waxes and phytochemicals, are also present (*Sousa et al.*, 2016). Natural honey is sticky,

viscous solution containing some 80–85% carbohydrates (mainly glucose and fructose), 15–17% water, 0.1–0.4% proteins, 0.2% ash and minor amounts of amino acids, enzymes, vitamins and other substances such as phenolic antioxidants (Buba *et al.*, 2013; Kek *et al.*, 2017).

The electrical conductivity is related to the concentration of soluble minerals, organic acids and proteins. It is a useful tool for discriminating honeys of different botanical origin (Acquraone *et al.*, 2007; Lazarević *et al.*, 2017). The storage time can also influence the electrical conductivity of honey (Karabagias *et al.*, 2014). The conductivity is very often used in routine quality control of honey. The electrical conductivity of honey is defined as that of a 20% weight in volume solution in water at 20° C, where the 20% refers to honey dry matter (International Honey Commission, 2009).

In accordance with the regulation concerning the quality of honey in the Republic of Serbia (*Official Gazette RS*, 101/2015), minimum electrical conductivity in honeydew put in the market is set to 0.8 mS/cm. For other types of honey, the maximum permitted value of electrical conductivity is 0.8 mS/cm.

The aim of the study presented in this paper was to investigate the electrical conductivity in various types of honey that were collected from the territory of the Republic of Serbia during two calendar years (2017 and 2018) in order to obtain information about honey quality, as well as to establish the relationship between electrical conductivity and botanical origin of honey.

Material and Methods

Honey samples

In total, 138 honey samples were collected from different retail chains in Serbia and Montenegro. Samples were collected as a part of official monitoring of honey and bee products quality during October 2017 in the Republic of Serbia and directly from honey producers from Serbia and Montenegro.

All collected samples were in their original packaging and transferred to the laboratory of Scientific Veterinary Institute “Novi Sad” for examination.

A total of 138 investigated samples included: 35 samples of acacia honey, 55 samples of meadow honey, 11 samples of floral honey, 13 samples of linden honey, 12 samples of honeydew honey, 7 samples of sunflower honey, 3 samples.

Table 1. Botanical source and number of samples of honey

Type of honey	No of samples 2017. year	No of samples 2018. year	Total number of samples
Meadow	18	37	55
Acacia	16	19	35
Linden	6	7	13
Multiflower	9	2	11
Honeydew	6	6	12
Sunflower	1	6	7
Rapeseed	-	3	3
Maritime	-	2	2

Electrical conductivity

Electrical conductivity was measured at 20° C in solutions of honey samples (20.0 g dry matter of honey in volume solution in 100 ml distilled water) using a conductometer Crison (Type Basic 30). The method of measuring is prescribed by International Honey Commission Methods (2009).

Statistical analysis was performed by PAST software package, version 2.12, Oslo, Norway. Data were grouped according to the type of honey and presented as mean \pm standard error, minimum and maximum values.

Results and Discussion

Average values of electrical conductivity of honey obtained in this study are summarized in Table 2.

Table 2. Results of determining electrical conductivity in diverse honey samples

TYPE OF HONEY	Total No. of samples/No of samples exceeded the limit	Electrical conductivity (mS/cm)		
		Referent values (Official Gazette RS, 101/2015)	Range	Average value \pm SD
Meadow	55/5	max 0.8	0.08-1.30	0.50 \pm 0.27
Acacia	35/0	max 0.8	0.08-0.80	0.21 \pm 0.14
Linden	13/2	max 0.8	0.53-1.03	0.75 \pm 0.13
Multiflower	11/0	max 0.8	0.09-0.74	0.50 \pm 0.18
Honeydew	12/3	min 0.8	0.09-1.99	1.04 \pm 0.48
Sunflower	7/1	max 0.8	0.28-0.85	0.50 \pm 0.19
Rapeseed	3/0	max 0.8	0.18-0.34	0.20 \pm 0.09
Maritime	2/0	max 0.8	0.59-0.85	0.70 \pm 0.18

The obtained values were compared with the values prescribed by the Regulation on the quality of honey in the Republic of Serbia (*Official Gazette, 101/2015*). The results were compared with the results from other authors from Serbia and other countries.

Electrical conductivity, closely related to the concentration of minerals and organic acids, showed a high variability within and between groups of honey. The values of electrical conductivity in the investigated honey samples were between 0.08 and 1.99 mS/cm. Out of a total of 138 tested honey samples, 11 samples did not comply with the local regulations for honeys (*Official Gazette, 101/2015*). Out of a total of 56 tested honey samples that were produced in the year 2017, 6 samples (10.7%) did not comply with the provisions of the Regulation, while of a total of 82 tested honey samples that were produced in the year 2018, 5 samples (6.1%) did not comply.

Within the group of meadow honey, electrical conductivity was above 0.8 mS/cm in 5 of 55 tested samples. In the group of linden honey, electrical conductivity was above 0.8 mS/cm in 2 from 13 tested samples. Within the group of forest honey, electrical conductivity value below 0.8 mS/cm was established in 3 from the total of 12 tested samples. Of the total of 7 tested sunflower honey samples, one sample did not comply with the local regulations for honeys.

The data indicated lowest conductivity in acacia honey samples, while the highest conductivity is found in samples of forest, maritime and linden honey. Similar values for electrical conductivity of honey are reported by other authors from our and other countries (*Vranić et al., 2017; Acquarone et al., 2007; Accorti et al., 1987; Boussaid et al., 2018; Chakir et al., 2016; Yadata, 2014; Kirs et al., 2011; Sousa et al., 2016; Escuerdo et al., 2014; Karabagias et al., 2014*).

In our research, the information about botanical origin was provided by beekeepers themselves. Therefore, potential inadequate estimation of the botanical origin of honey should be taken into consideration, thus, the samples that were not in accordance with the regulatory provisions might have not been properly classified into the relevant group of honey. *Kek et al. (2017)* suggested that the electrical conductivity of honey can vary by its different geographical, botanical and even entomological origins. Electrical conductivity is related to the ash content. Higher ash (minerals) content indicates higher electrical conductivity of honey. Minerals are introduced into honey with the pollen. The mineral and ash content therefore depends on the predominant pollen present in honey (*Kropf et al., 2008*). For the purpose of more reliable confirmation of the botanical origin of honey besides physico chemical analyzes, it is necessary to perform pollen analysis of honey (*Puusepp and Koff, 2014*). There are many reasons why it is important to determine the botanical origin of honey. The legislation requires the correct description of honey labels. The name of honey has to be characterized by specific blossom or

plant it originates from in such a way that, if there is any reference to a particular blossom or plant, the honey has to come wholly or mainly from that blossom or plant (*Vidaković et al., 2017*).

Conclusion

In conclusion, the result of this study indicated that honey samples from Serbia, were mostly of good quality in view of electrical conductivity. Electrical conductivity is a useful tool for discriminating honeys of different botanical origin. For the purpose of more reliable confirmation of the botanical origin of honey it is necessary to perform pollen analysis of honey.

A compliance with essential precautions is of vital importance to ensure standardization of beekeeping techniques, manufacturing and storing procedure as well as to improve honey quality. Therefore, further research and appropriate education of beekeepers is needed.

Električna provodljivost različitih vrsta srpskog meda

Milica Živkov Baloš, Sandra Jakšić, Nenad Popov, Suzana Vidaković, Dragana Ljubojević Pelić, Jasna Prodanov Radulović, Željko Mihaljev

Rezime

U ovom radu ispitivana je električna provodljivost različitih vrsta meda koji je prikupljen sa područja Republike Srbije, tokom dve kalendarske godine. Električna provodljivost može biti vrlo dobar kriterijum botaničkog porekla i čistoće meda i ima značajnu ulogu u definisanju ukupnih svojstava meda i proceni kvaliteta meda. Ispitano je ukupno 138 uzoraka meda različitog botaničkog porekla. Od ukupnog broja ispitanih uzoraka 11 uzoraka nije ispunilo zahteve Pravilnika Republike Srbije o kvalitetu meda, zbog neodgovarajuće električne provodljivosti. Izmerene vrednosti za električnu provodljivost kretale su se u opsegu od 0,08 and 1,99 ms/cm. Prema podacima našeg istraživanja najniža provodljivost utvrđena je kod uzoraka bagremovog meda i meda od uljane repice, dok su najvišu provodljivost imali uzorci šumskog i lipovog meda. Našim ostraživanjem potvrđeno je da je električna provodljivost korisna analiza za razlikovanje meda različitog botaničkog porekla. Radi pouzdanije potvrde botaničkog porekla potrebno je izvršiti i polensku analizu meda.

Ključne reči: med, električna provodljivost

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A COMPARISON OF TWO METHODS FOR DETERMINATION OF HMF IN HONEY: HPLC METHOD VERSUS SPECTROPHOTOMETRIC METHOD

Aleksandra M. Tasić, Tijana D. Mitrović, Marija Pavlović, Jasna Kureljušić

Institute of Veterinary Medicine of Serbia - Vojvode Toze 14, 11000 Belgrade, Serbia

Corresponding author: Aleksandra Tasić, alekstasic79@gmail.com

Original scientific paper

Abstract: In this paper the results of control of the quality of honey on the market of the Republic of Serbia are given by controlling the quantity of one of the most important control parameters, which is the content of 5-hydroxymethyl-2-furfural (HMF). Analysis of honey samples was carried out using two methods: high performance liquid chromatography (HPLC) and spectrophotometry method. It is important to note that the quality of honey, and in this connection, the HMF content in the honey, is influenced by a number of factors, such as processing conditions, the manner and period of packaging of honey, as well as the place of storage up to its consumption. The analysis of the results shows a large percentage of honey samples that had a permissible amount of HMF, but in the analysis there was a small number of samples that did not satisfy the mentioned quality parameter. The aim of study was developing two methods for determination of HMF; HPLC and spectrophotometric method and comparing the results obtained for the certified reference material. The results of the test showed good agreement of the results and high accuracy of the results obtained using both methods for determining HMF in the honey.

Key words: honey, HMF, HPLC, spectrophotometry

Introduction

Food products are subjected to heat treatment during cooking, such as cooking, pasteurization or sterilization, etc. and all these processes are commonly used to obtain the desired sensory or texture characteristics, or to eliminate microbiological as well as enzymatic activity. For some types of products, including milk or fruit juices, these modifications must be reduced as much as possible to achieve a natural and fresh appearance and taste. It is important to

emphasize that when these thermal processes occur during food conservation, they affect quality. During the thermal processing of food products, the so-called Millard reaction occurs, which includes reactions of amino acids, peptides and protein with reducing sugars and other carbonyl compounds. A chain of complex, competitive and successive reactions leads to the simultaneous development and creation of an intermediate whose interpretation and control is important for assessing the quality of (Woo *et al.*, 2015). Furan and its derivatives are one of the class compounds that can be found in many foods and beverages as a result of thermal treatment. To detect the intensity of thermal treatment of foods, the concentration of some indicators such as 5-hydroxymethyl-2-furfural is usually monitored. It is precisely this parameter that is equally important for controlling the quality of honey and monitoring its thermal processing. Its presence may be an indication of the spoilage, adulteration or heat stress of the sugar containing materials. Codex Alimentarius defines honey as the natural sweet substance produced by honeybees from the nectar of blossoms or from secretions of living parts of plants or excretions of plant sucking insects on the living parts of plants, which honeybees collect, transform and combine with specific substances of their own, store and leave in the honey comb to ripen and mature. The honey consists of a mixture of sugars, mainly fructose (~ 38.5%) and glucose (~ 31.0%), but also contains maltose, sucrose and other complex carbohydrates in a small amount. The honey also contains other substances in a small percentage, such as minerals (calcium, copper, iron, magnesium, phosphorus and potassium), proteins, amino acids, vitamins, flavonides, pigments, organic acids, as well as antioxidant compounds such as are chrysine, pinobanksin, vitamin C, catalase, and pinocembrin (Tette *et al.*, 2016). The substances mentioned in the honey are added to the bees, some of which originate from the honey plant, and some substances occur during the ripening of the honey. The specificities characterized by each honey, i.e. the origin of flowers, aroma, color and texture, predominantly depends on the type of flowers or plants from which the bees take nectar or butter, or geographical and botanical origin. Depending on the botanical origin of honey, there is a great variation with different flavors and colors, and variations in nectar content, together with other factors such as climatic conditions, soil type and bee activity, contribute to the existence of different types of honey (Bogdanov, 2009).

This paper provides an overview of the methods used for determining HMF, as well as the results obtained by monitoring the quality of honey over a longer period of time based on the analysis of the HMF content in different honey types, and the comparative use of two methods for determination.

Materials and Methods

Honey samples were stored at room temperature ($20\text{ }^{\circ}\text{C} \pm 4\text{ }^{\circ}\text{C}$) prior to analysis. The samples were collected from different local markers in Republic of Serbia under different conditions of storage. Ten grams of honey samples were dissolved in 25 ml deionized water and transferred quantitatively to a 50 ml volumetric flask, diluted to 50 ml with deionized water, filtered through a $0.45\text{ }\mu\text{m}$ membrane filter before analysis. The standard HMF is the analytical purity of the producer Dr Ehrenstorfer GmbH. Methanol is also analytical purity for HPLC determination by PanReac AppliChem.

For the chromatographic analysis, the Wathers system, consisting of autosempler and UV detector (1525 binary HPLC pump, 2487 Dual λ absorption detector, 717 Plus autosempler) was used. Chromatographic separation was performed using the Zorbax EclipsePlus C18 ($3.5\text{ }\mu\text{m}$, $3.6\text{ mm} \times 150\text{ mm}$) chromatography column. For the mobile phase, a solvent mixture was used: deionized water-methanol (90:10, v/v) at a flow rate of 1 ml/min. Samples of standards and analyzed samples were injected in an amount of 20 μl . For calibration of HMF, the following concentrations of the standard were prepared: 1, 2, 5 and 10 mg/l. Caffeine was used as internal standard (IS), and stock solutions (100 mg/l) were prepared by dissolving 10 mg of caffeine in 100.0 ml of deionised water and stored it at $4\text{ }^{\circ}\text{C}$ until analysis. The internal standard was used to optimize the method and conditions of the chromatogram. The standard working solutions were prepared everyday.

The absorbance of the solutions at the wavelength of 285 nm was determined using spectrophotometer (LLG, uniSpec2, UV-Visible). The quantitative value of HMF was determined using the proposed formula for the method (*Bogdanov, 2009*).

Results and Discussion

Physico-chemical parameters such as pH, acidity and electrical conductivity are considered important factors for quality control, as well as differentiation and in a certain way the classification of honey by species. One of the most important considerations regarding the quality of honey is the determination of the transformation product, such as the presence of hydroxymethylfurfural, which is primarily due to the decomposition of monosaccharides, mainly fructose. HMF is not present in fresh honey, but its concentration increases due to the conditions of storage of honey, excessive heating and higher age of honey. International standards for honey quality are listed in the European Honey Directive 001/110/EC and the Codex Alimentarius standard for

honey. In the case of fresh honey there is practically no HMF, but its content increases after storage, and the rate of production depends on the acidity of the honey and the storage temperature. In controlling honey, in general, the highest percentage of unsatisfactory honey is due to an increase in the amount of HMF and a decrease in enzyme activity of the diastase. The retention time for the standard hydroxymethylfurfurals and the analyzed honey samples at a flow rate of 1 ml/min is 9.171 minutes (Figure 1).

The mean value for the HMF content was between 1 and 10 mg/kg, which is an indicator of high quality honey, because in many papers and research for high quality honey is considered honey with a HMF value up to a maximum of 15 mg/kg (Table 1). The sample limit of detection (LOD, 0.15 mg/kg) and limit of quantification (LOQ, 0.5 mg/kg) were established by preparing the standards in a blank matrix.

In samples with unsatisfactory quality, the mean HMF value was over 100 mg/kg, or in the range of 60 to 315 mg/kg. These tested samples were overheated due to the de-crystallization process. The hydroxymethyl furfural is an aldehyde and a furan compound that is formed during the thermal decomposition of sugars and carbohydrates. The concentration of HMF in these samples was calculated reference to a linear regression analysis data or from a direct sample/standard comparison method.

The standard HMF area–concentration plot was linear over the range 1-10 mg/l with correlation coefficient (r) value 0.9998, slope (B) 151231 and intercept (A) 71022. The obtained results in mg/l were converted to mg/kg for honey samples. The International Honey Commission sets a limit of 80 mg/kg of HMF in honey. The rate of formation of HMF depends on the ratio of the present fructose and glucose as a form of sugar, since at a pH of 4.6 fructose has a five times higher glucose reactivity, so that a high fructose / glucose ratio will accelerate the reaction (*Shapla et al., 2018*). In addition, there are metal ions in the honey: manganese, zinc, magnesium and iron that have an effect on the formation of HMF. The percentage of metals Ca, P, Na, K, Fe, Zn, Cl and Mn ranged between 0.35% - 0.2%, 0.2% - 0.1%, 0.7% - 0.54%, 2.3% - 1.2%, 0.87% - 0.7%, 0.105% - 0.089%, 0.205% - 0.11% and 0.099% - 0.08%, respectively (*Suliman et al., 2013*). Recently it has also been shown that some substances presented in food may work as catalysts that enhance HMF and other furan aldehyde concentrations in food products. Preliminary studies in this field clearly show that in model systems multivalent metal cations including calcium and magnesium increase the yield of HMF (*Kowalski et al. 2013*).

HMF quantitative testing is conducted for juices where the results range from 0-75 mg/l. Indicators for good production and proper storage of juices are low levels of HMF and furosine, which is used as a common quality marker. Higher

amounts of furosine and insignificant amounts of HMF are considered indicators of prolonged storage, while high values of HMF and lower furosine may indicate serious heat treatment during the production of (Makawi *et al.*, 2009). Also, HMF values for sampled samples ranged between 26-807 mg/kg. High sugar content of these samples, together with heat treatment, can be the main cause of such a high value. It should be noted that in the legislation there are no restrictions on the content of HMF in jams.

Coffee is one of the most common drinks for which it is confirmed to contain HMF. The concentration of HMF in coffee depends on the process of frying and the type of coffee as well as the amount of sugar to be added. Before cooking, HMF levels for traditional and instant Turkish coffee samples were obtained between 213.02-238.99 and 336.03-362.05 mg/kg, respectively. However, after cooking, the HMF concentration increased 32.29-55.83% for the instant and 74.12 -224.75% for the traditional coffee (Mortas *et al.*, 2017). The methods established by the International Honey Commission allow for the parallel determination and control of HMF content using the spectrophotometric method and high pressure liquid chromatography.

Table 1: Results obtained for the HMF content in natural honey sample

Type of honey	Mean value of HMF content, mg/kg	Min value, mg/kg	Max, mg/kg
Acacia honey	8.1	0.5	14.8
Meadow honey	6.4	0.7	17.5
Sunflower honey	3.0	0.5	5.4
Polyflower honey	7.8	0.7	11.5

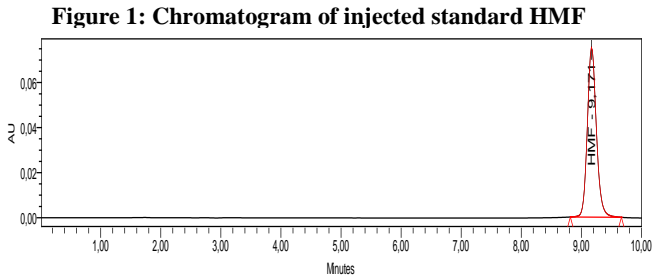


Table 2: The results obtained for the content of HMF in certified reference material of honey sample

Validation parameters	HMF, Spectrophotometric method	HMF, HPLC method
Means of the levels (mg/kg)	16.12	15.83
Standard deviation (SD) of repeatability of the level (mg/kg)	0.08	0.06
Relative standard deviation (RSD) of repeatability of the level (%)	0.49	0.40

The comparability of two methods was examined on certified reference material (Fapas, UK) in the Table 2. The assigned value of the certified reference material was 16.94 mg/kg, and the allowed range of the exact result was from 13.40 to 20.48 mg/kg. Results of measurements show lower levels of HMF using HPLC method (Table 2). The reason is probably matrix effect using various method and measurements at various spectrophotometric detectors.

Table 3: Results obtained for the HMF content in natural honey sample using two different methods

Type of honey	Mean value of HMF content, mg/kg	HMF, Spectrophotometric method	HMF, HPLC method
Acacia honey	7.49	7.64	7.34
Meadow honey	4.82	4.89	4.75
Sunflower honey	1.31	1.32	1.30
Polyflower honey	5.34	5.34	5.35

Table 3 gives the results of individual honey samples that have been used by both methods. In general, it can be said that there is not much difference in the results obtained for the quantitative HMF parallel use of both methods of determination, and HPLC and spectrophotometric methods. Which means that both methods can be used for routine testing and research in laboratory conditions using commercial honey samples.

Conclusion

It can be concluded that honey produced in the Republic of Serbia is of good quality. When the process of cultivation of honey is carefully controlled, it is

inevitably obtained even in high quality. Therefore, adequate training and good beekeeping practices are crucial to obtaining adequate products that are expected both by consumers and by legislation. This study is useful and crucial for understanding the local characteristics of honey and very important for the commercialization of regional honey.

Poređenje dve metode za određivanje HMF u medu: HPLC metode naspram spektrofotometrijske metode

Aleksandra M. Tasić, Tijana D. Mitrović, Marija Pavlović, Jasna Kureljušić

Rezime

U ovom radu dati su rezultati kontrole kvaliteta meda na tržištu Republike Srbije, kroz kontrolu količine jednog od najvažnijeg parametra kontrole, a to je sadržaja 5-hidroksimetil-2-furfurala (HMF-a). Analiza uzoraka meda sprovedena je korišćenjem dve metode: tačne hromatografije visokih preformansi (HPLC) i spektrofotometrijske metode. Važno je istaći da na kvalitet meda, a sa tim u vezi i na sadržaj HMF u medu, utiče veliki broj faktora kao što su uslovi obrade, način i period pakovanja meda, kao i mesto skladištenja do njegovog konzumiranja. Analiza rezultata pokazuje veliki procenat uzoraka meda koji su imali u granicama dozvoljenu količinu HMF, ali je prilikom analize bio i neznatan broj uzoraka koji nije zadovoljio pomenuti parametar kvaliteta. Cilj ispitivanja bio je razviti dve metode za određivanje HMF; HPLC i spektrofotometrijsku metodu i poređenje rezultata dobijenih krišćenjem sertifikovanog referentnog materijala. Rezultati ispitivanja pokazali su dobro slaganje rezultata i visoku tačnost rezultata dobijenih korišćenjem obe metode za određivanje HMF u medu.

Ključne reči: med, HMF, HPLC, spektrofotometrija

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CARBOHYDRATE AND PROTEIN FRACTIONS, AND FERMENTATION CHARACTERISTICS OF COMMON VETCH – OAT SILAGES

Jordan Marković, Tanja Vasić, Dragan Terzić, Dragoslav Đokić, Jasmina Milenković, Mladen Prijović, Đorđe Lazarević

Institute for Forage Crops Kruševac, 37251 Globoder, Republic of Serbia
Corresponding author: Jordan Marković, jordan.markovic@ikbks.com
Original scientific paper

Abstract: The objective of this study was to determine the fermentation characteristics of common vetch – oat silages, as well as carbohydrate and protein fractions of silage depending on different seeding rate in the mixtures and application of bacterial inoculant. The present experiment was conducted at the experimental field of the Institute for forage crops, Kruševac, Republic of Serbia. The common vetch and oat mixtures were ensiled with bacterial inoculant (a_1) and without bacterial inoculant (a_2). The common vetch and oat were tested at five different mixture rates: b_1) 100% common vetch + 0% oat; b_2) 0% common vetch + 100% oat; b_3) 25% common vetch + 75% oat; b_4) 50% common vetch + 50% oat and b_5) 75% common vetch + 25% oat. Carbohydrate and protein fractions by CNCPS (Cornell Net Carbohydrate and Protein System) and fermentation characteristics of silages were determined. Application of bacterial inoculant increased significantly intermediately degradable total carbohydrates (CB_2), non-protein nitrogen (PA) and intermediately degraded protein (PB_2) fraction, but decreased water soluble nitrogen content, acetic acid content, slowly degradable total carbohydrates (CB_3) and bound protein (PC) fraction. Pure common vetch silage had the highest CB_2 and the lowest CB_3 and the highest PA fraction of CP. Pure oat silage had the highest water soluble nitrogen and total carbohydrates (CHO) content, CB_3 and PC fraction, but the lowest content of undegradable CHO (CC) fraction, crude protein (CP) content and PB_2 fraction.

Key words: common vetch – oat silages, protein fractions, carbohydrate fractions

Introduction

Common vetch is an annual legume crop mostly used in mixtures or intercropping with cereals. In mixtures, oat provides structural support for common vetch growth, improves light interception and facilitate mechanical harvest, whereas common vetch in mixtures improves the quality of forages (*Lithourgidis et al., 2006*). Despite its relatively low crude protein concentration, oat is regarded as an important forage crop because of both high yields and high carbohydrate levels (*Erol et al., 2009*).

One anticipated advantage of feeding bi-crop silages of cereals and legumes is an improvement in the efficiency of nutrient utilization due to the possible synchronous supply of readily fermentable energy and protein in the rumen (*Adesogan et al., 2002*). Detailed information about protein and carbohydrate fraction in the ruminant diets which enable maximal utilization of present nutrients is necessary for ruminant feeding. In companion crops consisting of legumes and cereals, the contents of protein and carbohydrate fractions depend on their ratio in the mixture, stage of development and fermentation quality (*Đorđević et al., 2018*). Cornell Net Carbohydrate and Protein System – CNCPS (*Fox et al., 2003*) is taking into account those variations in protein and carbohydrate components when calculating metabolizable energy and in order to maximize microbial protein synthesis when formulating rations for dairy cows.

Experience with cultivation of legume-cereal mixtures for silage is limited, and there have been no enough studies on different mixtures for this purpose. The aim of the present study was to evaluate the fermentative characteristics of common vetch-oat silages, as well as protein and carbohydrate fractions of silages depending on different seeding rate in the mixtures and application of bacterial inoculant.

Materials and Methods

Common vetch and oat were grown in binary mixtures at the experimental field of the Institute for forage crops, Kruševac – Serbia (21° 19' 35" E, 43° 34' 58" N). The experiment was designed with three replication according to a randomized complete block. Experiment was established in autumn in 2014, on October the 20th. The common vetch and oat mixtures were ensiled with bacterial inoculant (a_1) and without bacterial inoculant (a_2). The common vetch and oat were tested at five different mixture rates: b_1) 100% vetch + 0% oat; b_2) 0% vetch + 100% oat; b_3) 25% vetch + 75% oat; b_4) 50% vetch + 50% oat and b_5) 75% vetch + 25% oat. All

mixtures were sown on plots of 20 m². Plant samples were taken in spring 2015, at forming green seeds in 2/3 pods of common vetch.

The vetch:oat mixtures were ensiled in the experimental containers holding 130 dm³, with three replications. After compaction, silomass covered with plastic wrap, and covered with a layer of sand thickness of about 10 cm as the main load. Bacterial inoculant *BioStabil Plus* which contained homo-fermentative lactic acid bacteria (*Enterococcus faecium* and *Bacillus plantarum*) and hetero-fermentative lactic acid bacteria (*Bacillus brevis*) with a concentration of 5x10¹⁰ CFU per gram was added, and ensiled in anaerobic jars for 45 days.

In order to estimate fermentation characteristics of silage the DM (Dry Matter) content by oven drying at 60° C for 48 h, the degree of acidity (pH), ammonia and soluble nitrogen, content of acetic, butyric and lactic acid were determined in the silage (Đorđević *et al.*, 2003).

The crude protein (CP) of the samples was determined using Kjeldahl method. The non-protein nitrogen (NPN), neutral detergent insoluble crude protein (NDICP), acid detergent insoluble crude protein (ADICP), soluble protein (SolP), true protein (TP) and insoluble protein (IP) were determined by Licitra *et al.* (1996). The cornell net carbohydrate and protein system (CNCPS) crude protein fractions of the samples, PA, PB₁, PB₂, PB₃ and PC were calculated based on CP, NPN, SolCP, NDICP, ADICP contents of samples according to Fox *et al.* (2004): PA = NPN; PB₁ = SolCP – NPN; PB₂ = CP – SolCP – NDICP; PB₃ = NDICP – ADICP; PB = 1000- PA – PC; PC = ADICP. Where, PA refers to the non-protein nitrogen (g kg⁻¹ DM); PB₁ the rapidly degraded crude protein (g kg⁻¹ DM); PB₂ the intermediately degraded crude protein (g kg⁻¹ DM); PB₃ slowly degraded crude protein (g kg⁻¹ DM) and PC the bound crude protein (g kg⁻¹ DM).

Total carbohydrates [CHO = 1000 – (CP + Ash + EE)] and non-fiber carbohydrates [NFC = 1000 – (NDF + CP + Ash + EE)] were calculating according to NRC (2001). Neutral detergent fiber (NDF), acid detergent fiber (ADF), hemicelluloses (HCL) and lignin content were determined according to Van Soest *et al.* (1991). WSC (water soluble carbohydrates – monosaccharides and disaccharides) were determined as water soluble carbohydrates according procedures described by Hall *et al.* (1999). Total CHO are divided into five fractions according CNCPS: instantaneously solubilizable CHO (CA, *i.e.* NSC); rapidly degradable CHO (CB₁, *i.e.* starch); intermediately degradable CHO (CB₂, *i.e.* NFC – NSC – Starch); slowly degradable CHO (CB₃, *i.e.* NDF – CC) and undegradable CHO (CC, *i.e.* NDF x (Lignin / NDF) x 2.4) composed of completely undegradable NDF (Lanzas *et al.*, 2007).

The fermentation characteristics of common vetch – oat silages, and protein and carbohydrate fractions by CNCPS are presented in this paper. The experimental data were analyzed by a two-way analysis of variance for silage

samples using a model that accounted for the main effects of common vetch – oat mixtures and addition of inoculants. Effects were considered significant at $p < 0.05$ level. The significance of differences between arithmetic means was tested by LSD test (STATISTICA 6, Stat. Soft. 2006).

Results and Discussion

Fermentation characteristics of common vetch – oat silages depending on the application of bacterial inoculant and seeding rate of common vetch in the mixture are presented in the Table 1.

The results of this investigation showed that bacterial inoculant did not affect significantly differences between treatments for DM, pH, ammonia nitrogen, butyric acid and lactic acid. Application of bacterial inoculant influenced lower values ($p < 0.05$) only for water soluble nitrogen (63.9% of total N *vc.* 65.6% of total N in treatment without bacterial inoculant) and for acetic acid content (45.0 g kg^{-1} DM *vc.* 54.1 g kg^{-1} DM in treatment without bacterial inoculant).

The different seeding rate of the common vetch in the vetch-oat mixtures influenced DM content of vetch-oat silages. The lowest numerical content of DM was found for the pure common vetch silage (325.2 g kg^{-1}), and higher content of DM was detected in pure oat silage (334.3 g kg^{-1}), but with increasing common vetch ratio in the mixture DM content increased. pH values of all investigated silages were numerical similar, and ranged from 4.47 in the ensiled mixture contained the highest common vetch seeding rate to 4.63 in the ensiled mixture contained the lowest common vetch seeding rate. Except for ammonia nitrogen content, all fermentation characteristics were affected by common vetch and oat seeding rate in the mixtures. Pure oat silage had the highest DM content ($p < 0.05$) and the lowest ammonia nitrogen content ($p < 0.05$), but it had the highest water-soluble nitrogen content ($p < 0.05$). The lactic acid content was the highest in pure common vetch silage (156.2 g kg^{-1} DM) and the lowest in the pure oat silage (91.9 g kg^{-1} DM). Increasing seeding rate of common vetch in the mixtures influenced increasing the lactic acid content in silages. Despite of this, acetic acid content was highest in 50:50 common vetch-oat silage, followed by pure oat silage, which were significantly differenced between each other ($p < 0.05$). Butyric acid was detected in all silages at low amounts, with no differences ($p > 0.05$) between pure common vetch and pure oat silage. Legumes as an important source of protein are very difficult to ensilage due to the high buffering capacity and low concentrations of easily soluble sugars. In mixture with grasses or cereals their fermentable characteristics are improved, so that silage of adequate quality can be obtained (Bijelić *et al.*, 2015). Dawson *et al.* (2002) by harvesting the plants at the later

stages, obtained silages with substantially higher content of acetic acid and ammonia nitrogen.

Table 1. Fermentation characteristics of common vetch – oat silages

		b ₁	b ₂	b ₃	b ₄	b ₅	
DM, g kg ⁻¹	a ₁	326.0 ^b	334.3 ^a	327.3 ^b	333.7 ^a	333.0 ^a	330.9^{NS}
	a ₂	324.3 ^b	334.3 ^a	327.3 ^b	333.3 ^a	334.0 ^a	330.7^{NS}
		325.2^B	334.3^A	327.3^B	333.5^A	333.5^A	
pH	a ₁	4.78 ^b	4.43 ^c	4.98 ^a	4.18 ^c	4.32 ^d	4.54^{NS}
	a ₂	4.37 ^d	4.60 ^b	4.27 ^c	4.95 ^a	4.61 ^b	4.56^{NS}
		4.58^A	4.51^B	4.63^A	4.57^A	4.47^B	
NH ₃ –N / Σ N % Σ N	a ₁	19.0 ^c	19.0 ^c	22.7 ^a	21.6 ^a	21.4 ^a	20.7^{NS}
	a ₂	22.6 ^a	19.8 ^b	18.4 ^c	20.6 ^b	19.3 ^b	20.1^{NS}
		20.8^A	19.4^B	20.5^A	21.1^A	20.3^A	
H ₂ O –N / Σ N % Σ N	a ₁	56.0 ^e	65.3 ^b	65.3 ^b	64.7 ^c	68.3 ^a	63.9^B
	a ₂	61.7 ^d	71.3 ^a	65.0 ^b	64.6 ^c	65.3 ^b	65.6^A
		58.8^C	68.3^A	65.1^B	64.6^B	66.8^A	
AA, g kg ⁻¹ DM	a ₁	45.6 ^d	42.7 ^c	50.3 ^c	49.3 ^c	37.0 ^f	45.0^B
	a ₂	50.8 ^c	59.4 ^a	46.6 ^d	58.4 ^a	55.3 ^b	54.1^A
		48.2^C	51.1^B	48.4^C	53.8^A	46.2^C	
BA, g kg ⁻¹ DM	a ₁	3.7 ^b	2.6 ^c	3.5 ^c	4.6 ^b	2.7 ^c	3.4^{NS}
	a ₂	2.6 ^c	4.0 ^b	2.3 ^c	5.6 ^a	5.6 ^a	4.0^{NS}
		3.2^C	3.3^C	2.9^D	5.1^A	4.1^B	
LA, g kg ⁻¹ DM	a ₁	140.0 ^b	96.9 ^e	97.7 ^c	122.6 ^c	121.3 ^c	115.7^{NS}
	a ₂	172.4 ^a	86.9 ^f	92.4 ^e	99.7 ^d	112.6 ^d	112.8^{NS}
		156.2^A	91.9^D	95.0^D	111.1^C	116.9^B	

a₁ – treatment with bacterial inoculant; a₂ – treatment without bacterial inoculant; b₁ – 100% common vetch + 0% oat; b₂ – 0% common vetch + 100% oat; b₃ – 25% common vetch + 75% oat; b₄ – 50% common vetch + 50% oat; b₅ – 75% common vetch + 25% oat; DM – dry matter; AA – acetic acid; BA – butyric acid; LA – lactic acid; Different letters denote significant difference means at the 5% level of significance.

The addition of bacterial inoculant decreased CA and CB₁ fraction of CHO with no differences between treatments, and CB₃ fraction (p< 0.05). The application of bacterial inoculant increased CB₂ fraction (p< 0.05) and CC fraction, but there were no differences between treatments (Table 2).

The highest content of CHO was recorded in pure oat silage (756.5 g kg⁻¹ DM) and decreased in common vetch-oat silages with decreasing seeding rate of oat in mixtures from 722.9 g kg⁻¹ DM to 684.1 g kg⁻¹ DM (p< 0.05). CB₂ fraction was lower in pure oat silage (288.1 g kg⁻¹ DM) than in pure common vetch silage (434.5 g kg⁻¹ DM), and its content increased with increasing common vetch proportion in common vetch – oat silages. Common vetch silage contained the lowest CB₃ fraction, and content of this fraction decreased from 402.7 g kg⁻¹ DM to 300.2 g kg⁻¹ DM (p< 0.05) with increasing common vetch proportion in common vetch – oat mixtures. Common vetch silage had higher content of CC fraction than

oat silage ($p < 0.05$), and the highest CC fraction was determined in silage with the highest seeding rate of common vetch in the mixture.

Table 2. Carbohydrate fractions of common vetch – oat silages

		b ₁	b ₂	b ₃	b ₄	b ₅	
CHO, g kg ⁻¹ DM	a ₁	656.2 ^g	758.0 ^a	722.2 ^b	699.4 ^d	677.7 ^f	702.7 ^{NS}
	a ₂	663.4 ^g	755.0 ^a	723.5 ^b	706.7 ^c	690.6 ^e	707.8 ^{NS}
		659.8^E	756.5^A	722.9^B	703.0^C	684.1^D	
CA, g kg ⁻¹ CHO	a ₁	39.1 ^d	42.1 ^d	35.6 ^e	59.4 ^b	69.5 ^a	49.1 ^{NS}
	a ₂	46.4 ^d	45.5 ^d	48.2 ^c	65.6 ^b	78.0 ^a	56.7 ^{NS}
		42.8^C	43.8^C	41.9^C	62.5^B	73.7^A	
CB ₁ , g kg ⁻¹ CHO	a ₁	47.0 ^d	54.8 ^c	45.7 ^c	42.5 ^c	69.5 ^a	51.9 ^{NS}
	a ₂	48.5 ^d	55.2 ^c	51.7 ^d	55.0 ^c	61.7 ^b	54.4 ^{NS}
		47.7^C	55.0^B	48.7^C	48.7^C	65.6^A	
CB ₂ , g kg ⁻¹ CHO	a ₁	439.3 ^a	315.4 ^b	263.0 ^d	301.1 ^c	312.7 ^b	326.3 ^A
	a ₂	429.7 ^a	260.7 ^d	282.0 ^d	248.1 ^e	249.8 ^e	294.0 ^B
		434.5^A	288.1^B	272.5^B	274.6^B	281.3^B	
CB ₃ , g kg ⁻¹ CHO	a ₁	252.7 ^f	378.4 ^c	403.9 ^b	369.9 ^c	287.4 ^e	338.5 ^B
	a ₂	255.1 ^f	446.5 ^a	401.4 ^b	396.2 ^b	313.0 ^d	362.5 ^A
		253.9^D	412.4^A	402.7^A	383.1^B	300.2^C	
CC, g kg ⁻¹ CHO	a ₁	221.9 ^d	209.3 ^e	251.8 ^b	227.0 ^d	260.8 ^b	234.2 ^{NS}
	a ₂	220.4 ^d	192.0 ^f	216.7 ^d	235.0 ^c	297.5 ^a	232.3 ^{NS}
		221.1^C	200.7^D	234.2^B	231.0^B	279.2^A	

a₁ – treatment with bacterial inoculant; a₂ – treatment without bacterial inoculant; b₁ – 100% common vetch + 0% oat; b₂ – 0% common vetch + 100% oat; b₃ – 25% common vetch + 75% oat; b₄ – 50% common vetch + 50% oat; b₅ – 75% common vetch + 25% oat; CHO – total carbohydrates; CA – instantaneously degradable CHO; CB₁ – rapidly degradable CHO; CB₂ – intermediately degradable CHO; CB₃ – slowly degradable CHO; CC – undegradable CHO; Different letters denote significant difference means at the 5% level of significance

Results of this investigation showed that common vetch silage had the highest CP content, followed by the silage 75:25 common vetch-oat mixture ($p < 0.05$). In contrast, pure oat silage had the lowest CP content. In all silages, the CP content increased as common vetch proportion in mixtures increased.

The addition of inoculants slightly increased CP content ($p > 0.05$), PA fraction ($p < 0.05$), PB₂ fraction ($p < 0.05$) and PB₃ fraction ($p > 0.05$), but decreased PC fraction ($p < 0.05$) and PB₁ fraction ($p > 0.05$). Analyses of CNCPS protein fractions in common vetch – oat silages showed that silage from common vetch monoculture contained the highest PA fraction. The highest PB₁ fraction was determined in pure oat silage, but content of this fraction increased with increasing common vetch proportion in the mixtures. PC fraction was also the highest in pure oat silage. *Blagojević et al. (2017)* investigated the influence of inoculation on protein fractions in companion crops of pea and oat. Authors detected that inoculation of companion crops resulted in significant decrease in PA protein fraction and significant increase in PB₁ and PB₃ fractions of crude protein.

Table 3. Protein fractions of common vetch – oat silages

		b ₁	b ₂	b ₃	b ₄	b ₅	
CP, g kg ⁻¹ DM	a ₁	204.0 ^a	91.1 ^e	117.3 ^d	138.0 ^c	164.0 ^b	142.9^{NS}
	a ₂	208.3 ^a	82.6 ^c	111.7 ^d	140.7 ^c	160.9 ^b	140.8^{NS}
		206.1^A	86.9^E	114.5^D	139.3^C	162.5^B	
PA, g kg ⁻¹ CP	a ₁	630.4 ^a	556.4 ^d	594.0 ^c	595.7 ^c	544.2 ^d	584.1^A
	a ₂	622.0 ^b	586.6 ^c	487.1 ^e	597.8 ^b	575.8 ^c	573.9^B
		626.2^A	571.5^B	540.5^C	596.8^A	560.0^B	
PB ₁ , g kg ⁻¹ CP	a ₁	27.5 ^b	35.6 ^a	20.7 ^c	23.0 ^b	26.7 ^b	26.7^{NS}
	a ₂	22.5 ^b	32.4 ^a	25.0 ^b	23.1 ^b	32.0 ^a	27.0^{NS}
		25.0^B	34.0^A	22.8^B	23.0^B	29.3^A	
PB ₂ , g kg ⁻¹ CP	a ₁	227.0 ^f	230.0 ^e	309.0 ^b	266.0 ^c	312.7 ^a	268.9^A
	a ₂	221.7 ^f	215.3 ^f	334.3 ^a	266.0 ^c	253.3 ^d	258.1^B
		224.3^D	222.7^D	321.7^A	266.0^C	283.0^B	
PB ₃ , g kg ⁻¹ CP	a ₁	27.0 ^c	24.1 ^d	19.7 ^d	25.7 ^d	46.3 ^a	28.6^{NS}
	a ₂	26.4 ^c	39.0 ^b	20.3 ^e	27.9 ^c	23.7 ^e	27.4^{NS}
		26.7^B	31.6^A	20.0^C	26.8^B	35.0^A	
PC, g kg ⁻¹ CP	a ₁	88.1 ^c	153.8 ^a	56.6 ^c	89.7 ^c	70.1 ^d	91.7^B
	a ₂	107.5 ^b	126.6 ^a	133.3 ^a	85.2 ^c	115.2 ^a	113.5^A
		97.8^B	140.2^A	94.9^C	87.4^C	92.6^C	

a₁ – treatment with bacterial inoculant; a₂ – treatment without bacterial inoculant; b₁ – 100% common vetch + 0% oat; b₂ – 0% common vetch + 100% oat; b₃ – 25% common vetch + 75% oat; b₄ – 50% common vetch + 50% oat; b₅ – 75% common vetch + 25% oat; CP – crude protein; PA – non-protein nitrogen; PB₁ – rapidly degraded protein; PB₂ – intermediately degraded protein; PB₃ – slowly degraded protein; PC – bound protein; Different letters denote significant difference means at the 5% level of significance.

Conclusions

In conclusion, bacterial inoculant application influenced lower content of water soluble nitrogen, acetic acid, CB₃ and PC fractions, but higher content of CB₂, PA and PB₂ fractions. The highest content of lactic acid was determined in common vetch silage. All silages showed satisfactory pH values which is indicative of well preserved silage and satisfactory content of lactic acid indicating a good fermentation. Higher content of ammonia nitrogen indicated that protein were extensively degraded. Common vetch silage had the highest CP, but also the highest content of PB₁. All investigated mixtures could be used for ruminant nutrition.

Ugljenohidratne, proteinske frakcije i parametri fermentacije silaža grahorice i ovs

Jordan Marković, Tanja Vasić, Dragan Terzić, Dragoslav Đokić, Jasmina Milenković, Mladen Prijović, Đorđe Lazarević

Rezime

Cilj ovih istraživanja je bio da se odredi kvalitet silaža grahorice i ovs, kao i sadržaj ugljenohidratnih i proteinskih frakcija silaža u zavisnosti od udela ovih komponenata u smeši i primene bakterijskih inokulanata. Ogled je postavljen na eksperimentalnom polju Instituita za krmno bilje u Kruševcu, Srbija. Grahorica i ovas su silirani uz primenu inokulanta – a_1 i bez inokulanta – a_2 . Istraživanja su obuhvatila pet različitih smeša: B_1) 100% grahorica + 0% ovas; B_2) 0% grahorica + 100% ovas; B_3) 25% grahorica + 75% ovas; B_4) 50% grahorica + 50% ovas; B_5) 75% grahorica + 25% ovas. Utvrđen je sadržaj proteinskih i ugljenohidratnih frakcija prema CNCPS sistemu i parametri fermentativnih procesa u silaži. Primena bakterijskih inokulanata je značajno uticala na povećanje sadržaja umereno razgradivih ugljenih hidrata (CB_2), neproteinskog azota (PA) i delimično razgradivih sirovih proteina (PB_2) frakcije, ali i smanjenje količine vodorastvorljivog azota, sirćetne kiseline, sporo razgradivih ugljenih hidrata (CB_3) i vezanih proteina (PC) frakcije. Silaža grahorice je imela najveći udeo CB_2 , a najmanji udeo CB_3 frakcije ugljenih hidrata, ali i najveći udeo PA frakcije sirovih proteina. Silaža ovs je sadržala najveću količinu vodorastvorljivog azota i ukupnih ugljenih hidrata, CB_3 i PC frakcije, ali najmanju količinu CC frakcije ugljenih hidrata, sirovih proteina i PB_2 frakcije sirovih proteina.

Ključne reči: silaža grahorice i ovs, proteinske frakcije, ugljenohidratne frakcije

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CROPPING SYSTEMS AFFECT PHOTOSYNTHETIC PIGMENTS AND GRAIN YIELD IN MAIZE

Vesna Dragičević, Milena Simić, Branka Kresović, Milan Brankov

Maize Research Institute “Zemun Polje”, Slobodana Bajića 1, 11185 Zemun Polje, Republic of Serbia
Corresponding author: Vesna Dragičević, vdragicevic@mrizp.rs
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Abstract: Maize is still widely growing in monoculture, due to its domination in sowing structure. Nevertheless, rotation, as cropping system has many advantages in regard to monoculture, revealing through better crop growth and yielding. The aim of this experiment was to evaluate different cropping systems, which include maize monoculture (M), maize-winter wheat-soybean (MWS) and maize-soybean-winter wheat (MSW) rotations, with different weed control measures: full and half of herbicide doses, weed removal by hoeing and control – without weed removal, on dry mass (DM), concentration of chlorophyll *a*, chlorophyll *b* and carotenoids in aboveground maize biomass, as well as grain yield during three seasons. Obtained results referred that DM accumulation in above ground biomass highly correlated with carotenoids concentration in maize leaves and that full dose of herbicides is an important strategy for greater DM accumulation. MSW is the most promising cropping system to achieve high grain yield of maize, what is possible tied to low variations in all three photosynthetic pigments, indicating that this system enables better conditions for assimilation and crop growth. Higher values of chlorophyll *a*, as found in treatment with weed removal, are important for maize productivity. What is more, carotenoids role is emphasized again, having primary role in chlorophyll *a* protection against oxidative stress, thus contributing to optimal assimilation and increased grain yielding potential.

Key words: grain yield, maize, crop rotation, monoculture, photosynthetic pigments, dry mass.

Introduction

Maize is still growing in monoculture in Serbia, due to its domination in sowing structure. Nevertheless, rotation, as cropping system has many advantages, when compared to monoculture, revealing through better crop growth and yielding. Predominantly two-crop rotation (winter wheat-maize) is present in sowing

structure in Serbia and it is followed by three-crop rotation (winter wheat-maize-soybean). It is well known that maize rotation with other crops could decrease number of pathogens, pests and weeds, maintain or increase soil fertility, enabling better conditions for maize growth and development (*Dolijanović et al., 2006; Stranger and Lauer, 2008; Riedell et al., 2009*). *Simić et al. (2016)* pointed out that three-crop rotation, such as maize-soybean-wheat, significantly reduced biomass of perennial and annual weeds, particularly when it is combined with standard herbicide application. This type of rotation, together with maize-wheat rotation was also positively reflected on grain yield increase. *Brankov et al. (2017)* likewise stated that herbicides expressed full effect in weed control in maize-wheat rotation and pointed that meteorological factors are very important for expression of herbicide and rotation effectiveness. Rotation provides optimal conditions for crop growth, increasing its competitiveness over weeds for resources such as light, water and nutrients (*Simić and Stefanović, 2007*). With such improved growing conditions it is able to apply lower amounts of herbicides in order to achieve high yields (*Simić et al., 2012*). Extended rotations that include maize and forage crops could reduce nitrogen inputs, together with increase in maize grain yield, giving them importance as more sustainable cropping systems over short-term rotations (*Stranger and Lauer 2008*). After 26 years of investigations, savings in agronomic inputs could be accomplished up to 30% when grain and silage maize were grown in rotation with different crops, together with increase of yield stability (*Borrelli et al., 2014*).

Spasojević et al. (2014) declared advantage of three-crop rotation which also included maize, soybean and wheat over maize monoculture with lower weed infestation, increased values of leaf area index, chlorophyll content, decreased free energy and carotenoids level, thus reflecting on increased grain yield. Similar results were obtained in maize-wheat rotation, when after three and five experimental years weed biomass was significantly decreased by rotation (averagely by 33.73%), as well as herbicide application, with negligible differences between recommended and half of recommended dose of herbicide. Parameters of maize competitiveness have been changed regarding to the herbicide level and they were greater in maize continuous cropping than in the maize-wheat rotation (*Simić et al., 2016*). Decrease in maize competitiveness could result from stress pressure, and it is associated with loss of or reduced synthesis of photosynthetic pigments, declined light harvesting and generation of reducing powers, having as a consequence reduction in dry matter accumulation, biomass and grain yield (*Jaleel et al., 2009*). Rationally cropped maize, i.e. applying of all necessary measures, primary rotation results in increased maize productivity, not only grain, but better dry matter accumulation with greater biomass (*Nevens and Reheul, 2001*).

The aim of this experiment was to evaluate different cropping systems, which include maize monoculture, maize-winter wheat-soybean and maize-soybean-winter wheat rotations, with different weed control measures - full and half of herbicide doses, weed removal by hoeing and control – without weed removal, regarding dry mass production, concentration of chlorophyll *a*, chlorophyll *b* and carotenoids of aboveground maize biomass, as well as grain yield during three seasons.

Material and Methods

The study was initiated in Maize Research Institute “Zemun Polje” in Serbia, with the aim to evaluate the effects of different cropping systems on maize growth and grain yield. The soil type at the experimental field was slightly calcareous chernozem with 53% sand, 30% silt, 17% clay; 3.3% of organic matter content and moderate drainage. The pH is 6.9 and soil structure is silty clay loam.

The following factors were evaluated: 1. maize cropping system - continuous cropping (M), maize-soybean-winter wheat (MSW) and maize-winter wheat-soybean (MWS) rotation; 2. weed control - herbicide mixture for complete control of broadleaf and grass weed species were applied at the recommended dose (H); half of recommended dose (1/2H); control, without weed removal and herbicide application (C); weed free, where weeds were removed by hoeing (WF).

Hybrid ZP606 was sown at the middle of April in 2012, 2015 and 2018. The standard technology of maize production was applied. Farmyard manure was applied in the autumn of previous year, in regard to growing season M and MSW system, while in MWS rotation farmyard manure was not applied. Additionally, mineral fertilizers were applied in the autumn and during early phases of maize development in the spring. The amounts of mineral fertilizers were determined by the soil analysis.

Every year, at the end of the pollination period and beginning of grain filling, when plants are completely developed, dry biomass of above-ground parts from three plants per elementary plot was measured. At the same time, cob leaves were sampled for analysis of photosynthetic pigments concentration: carotenoids, chlorophyll *a* and chlorophyll *b*, by the method of *Sarić et al. (1990)*. Grain yields were analysed at the end of the growing season. The maize grain yield was measured from two inner rows of each subplot and calculated to 14 % of moisture. The experimental data were statistically processed by analysis of the variance (ANOVA) and analysed by the LSD-test (5%), as well as correlation (Pearson correlation) and Principal Component Analysis (PCA) performed by SPSS 15.0 for Windows Evaluation version.

Meteorological conditions. Experimental years were relatively similar in precipitation level and monthly average temperature. The year 2012 was characterised with the lowest precipitation level (216.1 mm) and the highest average temperature (21.1 °C) compared to 2015 and 2018 (Table 1). 2012 and 2015 were also characterised with the unequal precipitation distribution, with only 4.8 and 7.2 mm amount in August of 2012 and July of 2015, signifying drought during grain filling period.

Table 1. Average monthly temperature and precipitation sum during seasons of 2012, 2015 and 2018

Year	April	May	June	July	August	September	October	Mean/Σ
Temperature, °C								
2012	14.4	17.9	24.6	27.1	26.2	22.1	15.4	21.1
2015	12.9	19.1	22.1	26.4	25.7	20.2	12.4	19.8
2018	18	21.7	22.7	23.6	25.7	19.8	15.9	21.0
Precipitation, mm								
2012	56.2	58.5	14.8	19.8	4.8	20.7	41.3	216.1
2015	19.7	97.8	31.1	7.2	56	73.6	65.1	350.5
2018	24.6	39	150.1	61.9	44	16.9	20.8	357.3

Results and Discussion

The significant variations in maize grain yield were obtained by the influence of all of the sources of variation and their interaction, while variations in concentration of carotenoids, chlorophyll *a* and *b* were mainly present under the influence of year and interactions of all examined factors (Table 2). Based on the average from all three years, the highest average grain yield was obtained in MSW cropping system (Figure 1). Also, 1/2H experimental variant was characterised by the significantly higher grain yields in all three cropping systems, with the highest value achieved in MSW-1/2H combination. WF systems had similar grain yield values, while M had the poorest results, as expected, particularly in C variant. Similarly, *Borrelli et al. (2014)* established that the grain yield gradually increased in rotation while the yield of maize grown in monoculture decreased slightly over 26 year period.

Table 2. Analysis of variance (LSD_{0.05} values) for the grain yield, concentrations of carotenoids chlorophyll b and chlorophyll a in maize leaves, under the influence of different cropping system, herbicide treatment and year

Source of variation	df	Grain yield	Carotenoids	Chlorophyll <i>b</i>	Chlorophyll <i>a</i>
		(t ha ⁻¹)	(µg g ⁻¹ DM)		
Replicate	3	LSD _{0.05}			
Cropping system (CS)	2	2.033*	4.202	2.821	8.236
Herbicide treatment (H)	3	1.975*	4.338	3.416	8.815
Year (Y)	2	1.709*	1.492*	2.074*	3.511*
CS × H	11	1.552*	1.351*	1.880*	3.054*
CS × Y	8	1.468*	1.408*	1.746*	2.725*
H × Y	11	1.552*	1.351*	1.880*	3.054*
CS × H × Y	35	1.184*	0.454*	0.349*	0.427*
Coefficient of variation (%)		1.05	0.15	0.91	1.36
Average		6.90	4.107	4.419	9.545
Min		2.660	0.381	0.801	0.535
Max		10.048	12.562	14.413	27.161

*Significant at 5% probability level; df: degrees of freedom; LSD: least significant difference.

In both three-crop rotations the highest DM concentration was present in H experimental variant (Figure 1), indicating importance of herbicides, as weed suppressants for productivity, i.e. dry matter accumulation in maize biomass, what is also supported by *Khan et al. (2012)*, who achieved the highest maize productivity on plots treated with herbicides. This statement was also supported with the highest DM values obtained in WF variant in M cropping system. This means that only in fields with proper weed control, maize plants are able to accumulate higher DM and eventually realise their full photosynthetic, as well as production potential. There were also present high fluctuations in DM concentration in M system, when compared to both rotation systems, confirming *Nevens and Reheul (2001)* statement that only rationally cropped maize, with applied rotations has increased DM in biomass.

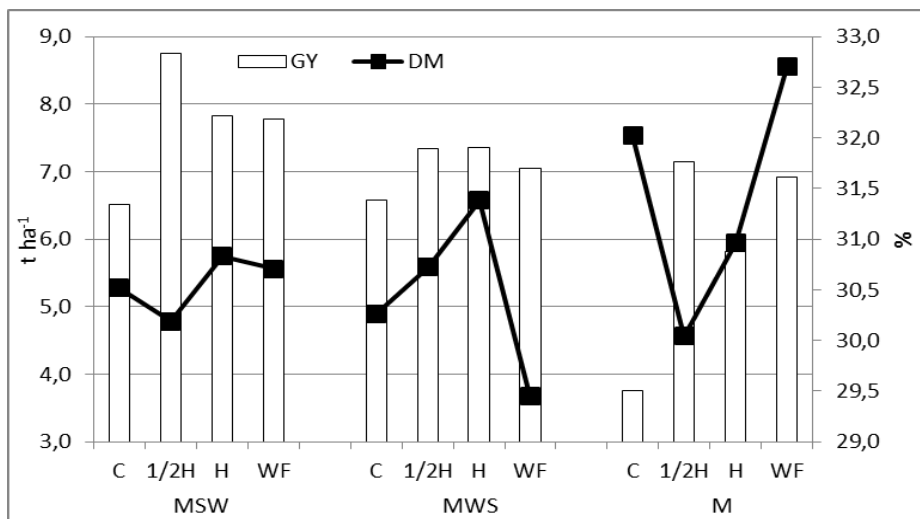


Figure 1. Average grain yield (GY) and dry mass of above-ground biomass (DM) of maize grown in different cropping systems (MSW – maize-soybean-winter wheat rotation; MWS – maize-winter wheat-soybean rotation; M – maize monoculture; C- control; 1/2H – half dose of applied herbicide; H – full dose of applied herbicide; WF – weed free)

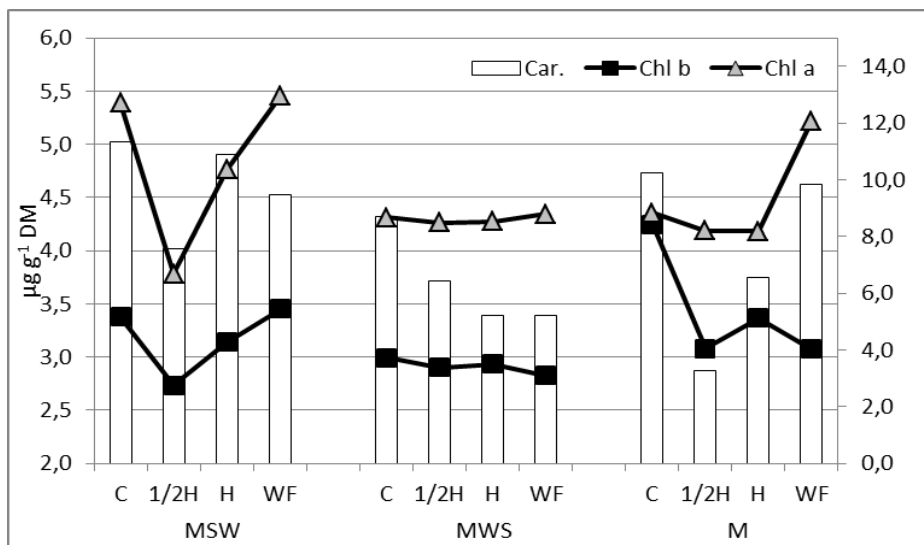


Figure 2. Average concentrations of carotenoids (Car.), chlorophyll *b* (Chl *b*) and chlorophyll *a* (Chl *a*) in leaves of maize grown in different cropping systems (MSW – maize-soybean-winter wheat rotation; MWS – maize-winter wheat-soybean rotation; M – maize monoculture; C- control; 1/2H – half dose of applied herbicide; H – full dose of applied herbicide; WF – weed free)

Opposite to productivity parameters (grain yield and DM concentration), concentration of photosynthetic pigments at the beginning of grain filling period had mainly the highest values in MSW three-field rotation (Figure 2). Nevertheless, relatively low variations in all three photosynthetic pigments was present in MWS cropping system, indicating that this system enables better conditions for plant growth and assimilation, irrespective to weed control measures. *Ennin and Clegg (2001)* also achieved higher productivity and chlorophyll content in maize grown after soybean, due to the higher N residues provided by soybean. Average concentration of carotenoids tend to be higher in control, evidencing about presence of stressful conditions, due to higher weed infestation (*Simić and Stefanović, 2007; Simić et al., 2012*). Carotenoids serve as an important protectors not only of photosynthetic apparatus against stress, but also of different plant tissues against free radicals produced in stressful conditions (*Jaleel et al., 2009*). This could be the reason of the significantly higher and positive correlation between carotenoids concentration and dry mass content (Table 3). When applied treatments for weed control were considered, the highest chlorophyll *a* concentrations was observable at WF treatment, in all three cropping systems, while the highest chlorophyll *b* concentration was present in control. Such situation indicates that optimal conditions for crop growth and increased potential for assimilation was enabled by weed removal with hoeing. However, increased overshadow and competitiveness between crop plants and weeds were present in control, similarly by results obtained on maize grown with different spatial arrangement (*Simić et al., 2012*). This was supported by significant and negative correlation between chlorophyll *b* concentration and grain yield, as well as significant and positive correlation between chlorophyll *b* and dry mass, evidencing that crop plants tend to accumulate greater dry biomass in conditions of overshadow (competitiveness) the rather than to translocate assimilative into grain, lowering yield potential (*Sarabi et al., 2011*).

Table 3. Correlation between grain yield and above-ground dry biomass content and concentrations of carotenoids, chlorophyll *b* and *a* in maize leaves

	Grain yield (t ha ⁻¹)	Dry mass (%)
Carotenoids	-0.206	0.429*
Chlorophyll <i>b</i>	-0.818*	0.471*
Chlorophyll <i>a</i>	-0.005	0.320*

Pearson correlation coefficients at the level of significance of 0.05*

Principal component analysis revealed that PC1 and PC2 participated with 53.3% and 25.8% in total variability, respectively. Grain yield and chlorophyll *b* mainly contributed to PC1, while carotenoids and chlorophyll *a* mainly contributed to PC2. According to data present in Figure 3, H, C and WF treatments from MSW

cropping system mainly induced variability in carotenoids and chlorophyll *a* concentration, while dry mass and chlorophyll *b* varied mainly in monoculture control (M-C). MWS cropping system (C, 1/2H, H and WF treatments), as well as MSW in combination with 1/2H largely contributed to the variation in grain yield, but in lesser extent. All that could indicate that high chlorophyll *b* concentrations are connected to lower yielding potential, as it was previously supposed, while carotenoids have primary role in chlorophyll *a* protection against oxidative stress, thus contributing to optimal assimilation and increased grain yielding potential (Jaleel *et al.*, 2009).

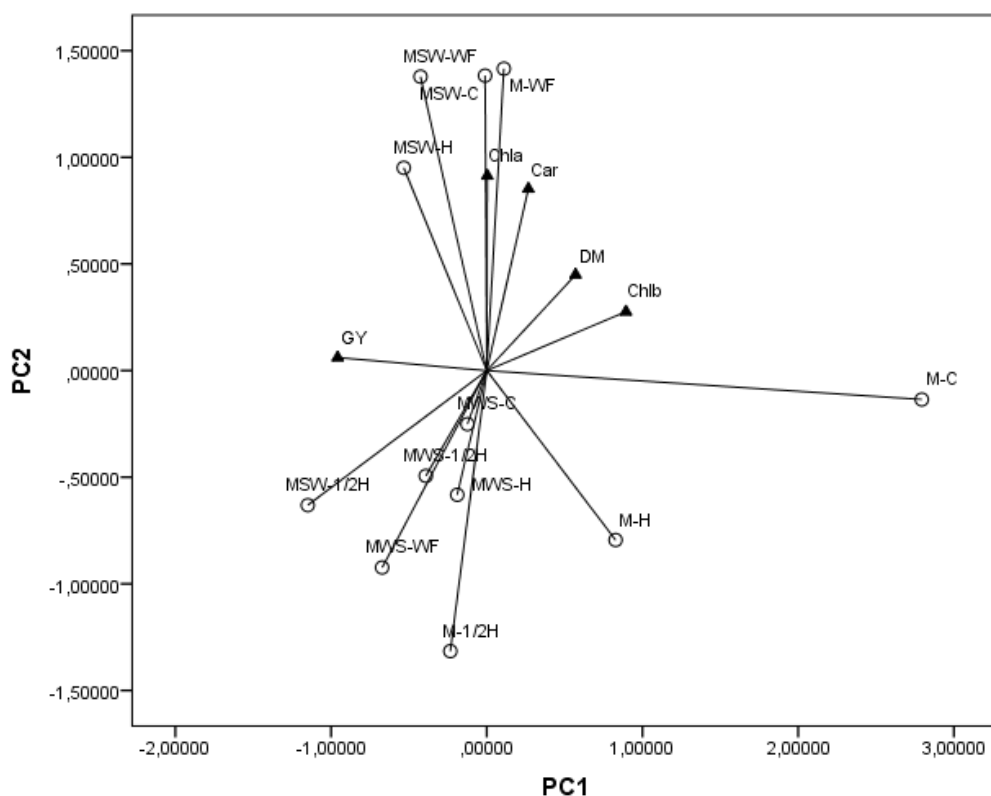


Figure 3. Principal Component Analysis for grain yield (GY), dry mass (DW), carotenoids (Car), chlorophyll b (Chl b) and chlorophyll a (Chl a) concentrations in leaves of maize grown in different cropping systems (MSW – maize-soybean-winter wheat rotation; MWS – maize-winter wheat-soybean rotation; M – maize monoculture; C- control; 1/2H – half dose of applied herbicide; H – full dose of applied herbicide; WF – weed free)

Conclusion

Obtained results referred that dry mass accumulation in above ground biomass depends on carotenoids concentration in maize leaves and that full dose of herbicides (H treatment) is an important strategy for greater DM accumulation. MSW is the most promising cropping system to achieve high grain yield of maize, what could be also tied to low variations in all three photosynthetic pigments, indicating that this system enables better conditions for plant growth and assimilation. Higher values of chlorophyll *a*, as found in WF treatment, are important for maize productivity. What is more, carotenoids role is emphasized again, having primary role in chlorophyll *a* protection against oxidative stress, thus contributing to optimal assimilation and increased grain yielding potential.

Kako sistemi gajenja utiču na fotosintetske pigmente i prinos zrna kukuruza

Vesna Dragičević, Milena Simić, Branka Kresović, Milan Brankov

Rezime

Najšire rasprostranjen način gajenja kukuruza je monokultura zahvaljujući njegovoj dominantnosti u setvenoj strukturi. Međutim, plodored ima brojne prednosti u odnosu na monokulturu koje se ogledaju u boljem rastu i prinosu useva. Cilj eksperimenta je da se uporede različiti sistemi gajenja koji uključuju monokulturu kukuruza (M), kao i rotacije kukuruz-ozima pšenica-soja (MWS) i kukuruz-soja-ozima pšenica (MSW), u kombinaciji sa različitim merama kontrole zakorovljenosti: puna i polovina doze herbicida, uklanjanje korova okopavanjem i kontrola – bez kontrole zakorovljenosti, na suhu masu (DM), koncentraciju hlorofila *a* i *b* i karotenoida u nadzemnoj biomasi kukuruza, kao i prinos zrna, na kraju vegetacije tokom tri sezone. Dobijeni rezultati ukazuju da akumulacija suve mase nadzemnih delova u visokom stepenu korelira sa promenama koncentracije karotenoida u listovima kukuruza i da je puna doza herbicida važna strategijac za veće nakupljanje suve mase. MSW se pokazao kao najperspektivniji sistem za povećanje prinosa zrna kukuruza, što je najverovatnije povezano sa smanjenjem u variranu fotosintetskih pigmenata, što upućuje da upravo ovaj sistem gajenja omogućava bolje uslove za asimilaciju i rast useva. Povećanje vrednosti hlorofila *a*, posebno u tretmanu gde su korovi ručno uklanjani su vrlo važne za produktivnost kukuruza. Važna uloga karotenoida je iznova istaknuta, preko zaštite

hlorofila *a* od oksidativnog stresa, što doprinosi optimalnoj asimilaciji i povećanju potencijala rodosti kukuruza.

Ključne reči: prinos zrna, kukuruz, plodored, monokultura, fotosintetički pigmenti, suva masa

Acknowledgment

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COMPETITIVE ABILITY OF SOYBEAN AND PROSO MILLET IN DIFFERENT INTERCROP COMBINATIONS

Milena Milenković, Milena Simić, Milan Brankov, Vesna Perić, Miodrag Tolimir, Vesna Dragičević

Maize Research Institute „Zemun Polje“, Slobodana Bajića 1, 11185 Zemun Polje, Serbia
Corresponding author: Milena Milenković, mmilena@mrizp.rs
Original scientific paper

Abstract: Intercropping includes cultivating of two or more crop species simultaneously on the same field. Benefits of intercropping can be achieved only if complementary crops are combined, which results in a higher and more stable yield in regard to sole crops. The competition of proso millet (M) and soybean (S) in different intercrop combinations (S-M, SS-MM, SS-MMMM), simultaneously testing influence of bio-fertilizer on them, was studied. In regard to competitive ratio and aggressivity, all combinations showed soybean as dominant and millet as dominated specie. Since SS-MM combination has significantly separated from other two, with high values of competitive ratio and aggressivity for soybean and low for millet, it can be concluded that ratio and planting pattern had significant influence on obtained results. Bio-fertilizer didn't express greater impact comparing with intercropping, which influence was much more pronounced. Soybean showed its highest competitive ability in SS-MM treated with Coveron (3.36), while average aggressivity for this combination was 0.95. In contrast to soybean, in this combination millet showed its lowest values. Anyhow, deeper studies are desirable to find out the most productive combination for forage biomass production.

Key words: soybean, proso millet, biomass, competitive ratio, aggressivity

Introduction

Intercropping is intrinsic part of the sustainable agricultural practice and it includes cultivating of two or more crop species simultaneously on the same field. It is an old and commonly used cropping practice that appeared as a solution for biodiversity disturbance commonly present in intensive agricultural systems. In intensive systems, high inputs of mineral fertilizers and pesticides have a negative impact on soil and water quality as well as biodiversity conservation. Therefore, combination of various measures applied in sustainable systems is used with the

aim to produce high quality and healthy crops together with preserving an agro-ecosystem.

Combining complementary crops in intercrop can provide better root interaction with available resources from soil, like as nutrients and microorganisms, and also to provide greater and more stable yield (*Dragičević et al., 2017; Lithourgidis et al., 2011; Malézieux et al., 2009*). Complementarity refers to the use of various resources in different time thus reducing their competitiveness. Plants compete with each other due to common requirements for space, light, depth rooting and nutrient and water uptake. Consequently, successful intercropping occurs when intercrop competition is less than intracrop competition (*Gebru, 2015*).

Cereals and legumes are recognized as favourable combination for intercropping because they are normally found in natural ecosystems (*Duchene et al., 2017*), improving soil fertility (N and P), enhancing micronutrients absorption and reducing damage caused by pests, diseases and weeds. Legumes have ability to fix atmospheric nitrogen and thus provide high protein content, while cereals are able to produce high dry matter yield and increase resistance to harmful conditions, which makes this combination effective for boosting forage biomass production, nutritional quality and monetary return (*Eskandari et al., 2009; Iqbal et al., 2018*). Usage of bio-fertilizer is additional way to encourage soil fertility and hence better exploitation of nutrients from soil. Results of *Dragičević et al. (2015)* confirmed that intercrop in combination with bio-fertilizer improved nutritional quality of maize and soybean grain without grain yield losses.

Proso millet (*Panicum miliaceum*) is an annual cereal rich in carbohydrates and therefore has high energy value. It is one of the most suitable crops for sustainable agriculture due to its unique characteristics such as drought and heat tolerance. In combination with soybean (*Glycine max* L.), this association can ensure better space utilization of intercrop than sole crops and consequently increase economic usage of land (*Ahmadvand and Hajinia, 2015; Habiyaemye et al., 2017*).

To evaluate possible advantageous of intercrop, compared to the pure crops, and to quantify beneficial competitive effects in different planting patterns, various indices are used (*Yang et al., 2017*). According to *Jahanzad et al. (2015)* land equivalent ratio (LER), competitive ratio (CR) and aggressivity (A) are the most common conventional methods for comparison, and they are used in this study.

Material and Methods

Aim of this study was to evaluate competitive ability of proso millet (*var.* Biserka) and soybean (*var.* Selena) in different intercrop combinations, simultaneously testing influence of bio-fertilizer, and to find the most perspective one. The experiment was carried out in Maize Research Institute „Zemun Polje“, Serbia, on slightly calcareous chernozem soil (pH 6.9), as a randomized complete block design, with four replications. The following intercrop combinations were tested and compared with sole crops: alternating rows of soybean and millet (S-M), alternating strips with 2 rows of soybean and 2 rows of millet (SS-MM) and alternating strips with 2 rows of soybean and 4 rows of millet (SS-MMMM). The effect of bio-fertilizer Coveron (containing *Glomus sp.* and *Trichoderma*; Italtollina, Italy) was also examined. Crops were sown at the beginning of May 2018, and experiment was managed in dryland farming. For above-ground green biomass determination plants were harvested in the early stage of maturity. Total precipitation in this period was 251 mm, and average temperature 22.7 °C.

The green biomass yield was measured and calculated in t ha⁻¹. Competition indices and intercropping efficiency such as land equivalent ratio (LER), competitive ratio (CR) and aggressivity (A) were calculated according to the formulas by Mead and Willey (1980) and Willey and Rao (1980).

Results were analysed using analysis of variance (ANOVA) and the significance of the treatments effect were determined by the Fisher's least significant difference (LSD) test at $p = 0.05$. The results are presented as mean with standard deviation (SD).

Results and Discussion

Based on LER values, previous results showed that the best land utilization was in SS-MM intercrop combination (Milenković *et al.*, 2019). When CR was calculated for soybean, significant difference appeared in SS-MM combinations (with and without Coveron) compared to the other combinations (Figure 1). In this situation, soybean has shown its highest competitive ability, especially in the treatment with bio-fertilizer (3.36). Also, it turned out that the influence of intercrop on competitive ability was much more pronounced than influence of bio-fertilizer, which was not significant.

In recent research it is proved that competitive behaviour of crops is different in various intercropping planting patterns which supports our results. Yang *et al.* (2017) proved that soybean can be dominant or dominated specie, in combination with maize, depending on the planting pattern of intercropping. They

just confirmed previous study where *Jahanzad et al. (2015)* showed how CR and A for millet and soybean can vary if is ratio 60M:40S or 60S:40M (in first one soybean is dominated and in second one soybean is dominant specie). In our research, results for soybean in SS-MM combination significantly differed from those for S-M and SS-MMMM, indicating dependence of species ratio and planting pattern on aggressivity and competitive ratio.

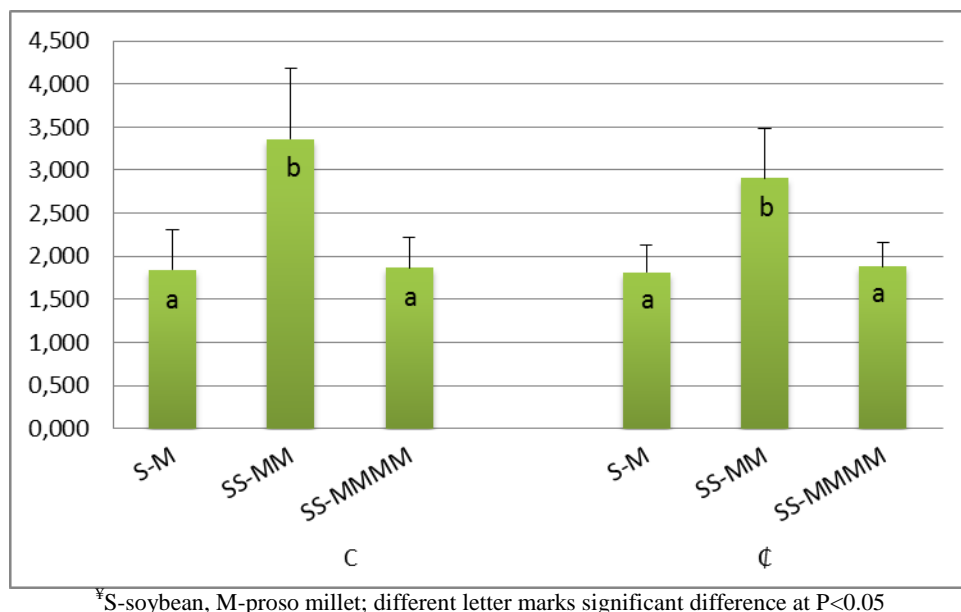
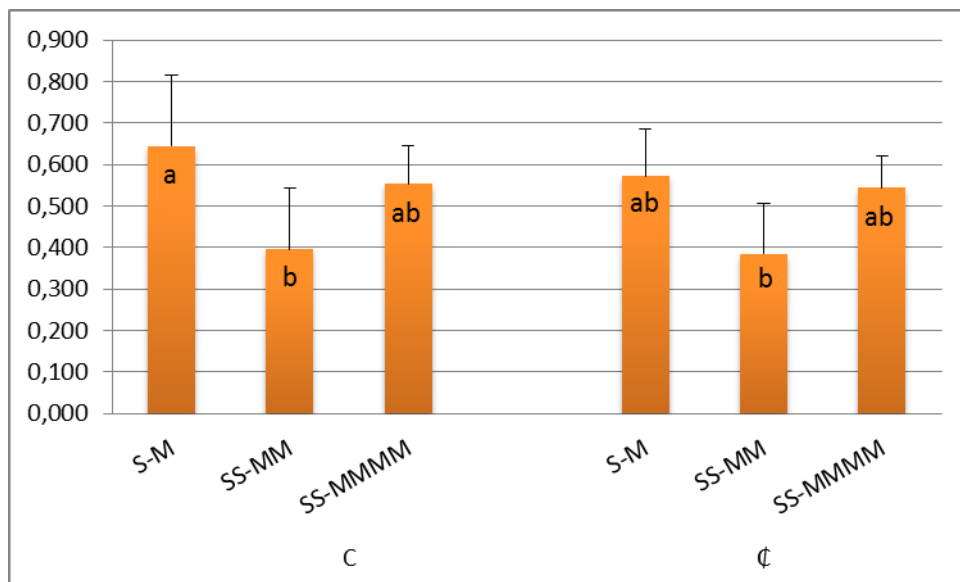


Figure 1. Competitive ratio (CR) with corresponding standard deviation for soybean in different intercrop combinations, with and without bio-fertilizer (C and C̄, respectively)

As expected, results for proso millet showed inversely results (Figure 2). For both species, SS-MM combination was singled out significantly, but in opposite terms. Where soybean was the most competitive, millet was depressed. Consequently, SS-MM had minimum values in comparison to the other four combinations (0.40 and 0.38, with and without Coveron, respectively). Intercropping affected this combination considerably in regard to the bio-fertilizer, especially influencing the difference between SS-MM and S-M combination. Taking into account combined effect of intercropping and bio-fertilizer, SS-MM are distinguished significantly from S-M combination treated with Coveron, but in regard to the other ones, difference didn't exist. From this situation it can be pointed that, even is the ratio of species the same, significant difference can occur. As is mentioned above, *Jahanzad et al. (2015)* showed that variations in species

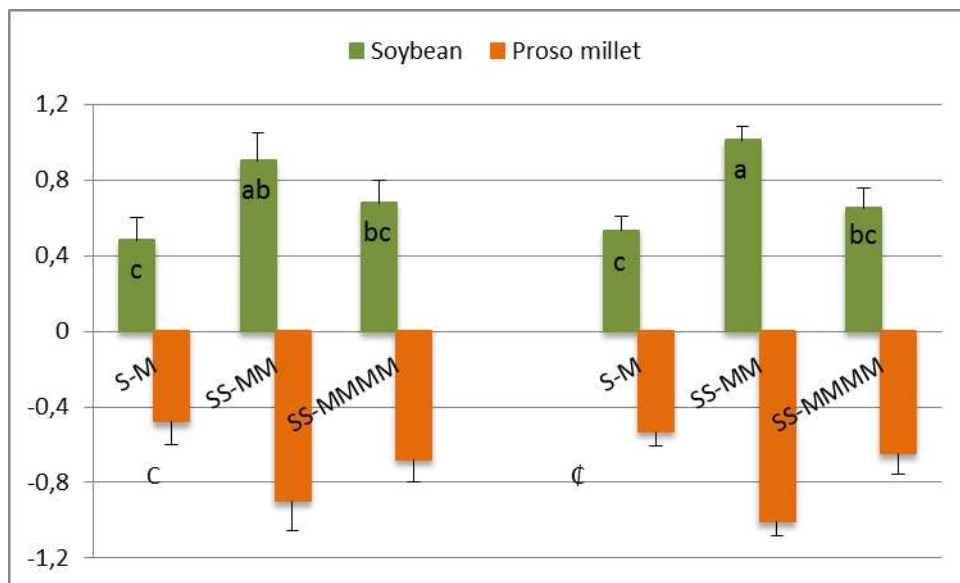
ratio make big difference in CR values, which requires additional research in order to find the most productive one for forage biomass production.



‡S-soybean, M-proso millet; different letter marks significant difference at $P < 0.05$

Figure 2. Competitive ratio (CR) with corresponding standard deviation for proso millet in different intercrop combinations, with and without bio-fertilizer (C and C̄, respectively)

Figure 3 shows that similar results to CR are obtained for soybean aggressivity in different intercrops. SS-MM intercropping combination, with average aggressivity of 0.95, significantly differed from S-M and SS-MMMM (0.50 and 0.66, respectively), while Coveron didn't expressed greater impact, again. Minor deviations between results for A and CR occur due to aggressivity presents simple difference between deviations of crops yields from their 'expected' yields, while CR better explain competitive ability comparing how much is one crop more competitive than the other one (Willey and Rao, 1980).



[†]S-soybean, M-proso millet; different letter marks significant difference at $P < 0.05$

Figure 3. Aggressivity (A) of soybean and proso millet with corresponding standard deviation in different intercrop combinations, with and without bio-fertilizer (C and Cf, respectively)

Results for millet aggressivity were the same as for soybean, but with negative values. In both situations Coveron didn't have greater influence on aggressivity. *Ahmadvand and Hajinia (2015)* in their research (with 25S:75M, 50S:50M and 75S:25M ratio) obtained results where proso millet was more dominate than soybean with positive values of aggressivity, which is in accordance with previous claim. These results indicate that many factors affect aggressivity and competitive ratio of soybean and proso millet species, and consequently require deeper research in order to obtain reliable and repeatable results.

Conclusion

In this study, in regard to aggressivity and competitive ratio, differences were observed between SS-MM combinations in regard to S-M and SS-MMMM. This combination singled out with the highest values for soybean and the lowest values for proso millet which classified the soybean as dominant and millet as dominated specie. All tested combinations had the same principle of competition. Moreover, it is shown that planting patterns and ratio in intercropping had major influence on results, while bio-fertilizer didn't have significant impact.

Kompetitivne sposobnosti soje i prosa u različitim kombinacijama združenih useva

Milena Milenković, Milena Simić, Milan Brankov, Vesna Perić, Miodrag Tolimir, Vesna Dragičević

Rezime

Združivanje useva obuhvata gajenje dve ili više vrste biljaka istovremeno na istom prostoru. Prednosti združivanja mogu se ispoljiti samo u slučajevima kada se kombinuju komplementarni usevi, što rezultira višim i stabilnijim prinosom u odnosu na samostalne useve. U ovom istraživanju ispitivana je kompeticija prosa (M) i soje (S) u različitim kombinacijama združenih useva (S-M, SS-MM, SS-MMMM), istovremeno prateći i uticaj bio-đubriva na njih. Što se tiče konkurentnog odnosa i agresivnosti, u svim kombinacijama soja je pokazala svoju dominantnost dok je proso bio izdominirana vrsta. S obzirom da se SS-MM kombinacija značajno izdvojila od ostale dve, sa visokim vrednostima konkurentnog odnosa i agresivnosti za soju i niskim za proso, može se zaključiti da su odnos biljaka i njihov prostorni raspored uticali na dobijene rezultate. Bio-đubrivo nije imalo velikog efekta u odnosu na samo združivanje, čiji se uticaj posebno istakao. Soja je pokazala svoju najveću kompetitivnu sposobnost u SS-MM kombinaciji tretiranoj Coveron-om (3.36), dok je prosečna agresivnost za ovu kombinaciju iznosila 0.95. Suprotno od soje, proso je iskazao svoje najniže vrednosti u navedenoj kombinaciji. U svakom slučaju, detaljnije studije su potrebne kako bi se pronašla najproduktivnija kombinacija za proizvodnju biomase.

Ključne reči: soja, proso, biomasa, konkurentni odnos, agresivnost

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MYCOPOPULATION OF DIFFERENT FABA BEAN GENOTYPES IN SERBIA

Tanja Vasić¹, Snežana Andjelković¹, Jordan Marković¹, Sanja Živković², Đorđe Lazarević¹, Mladen Prijović¹

¹Institute for Forage Crops, 37251 Kruševac, Republic of Serbia

²Faculty of Agriculture, University of Niš, Kosančićeva 4, 37000 Kruševac, Republic of Serbia

Corresponding author: Tanja Vasić, tanjavasic82@gmail.com

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Abstract: Faba bean (*Vicia faba* L.) is annual plant from the legume family (Fabaceae) and it originates from the temperate zone of Africa and Asia. It has a special place in the provision of animal feed (fodder) in the zone of moderate climate. It belongs to high-quality protein fodder plants.

There has not been a systematic research of faba bean mycoflora in Serbia. This research aims to present the results of preliminary research of mycopopulation of 10 different genotypes of faba bean. Total of 400 plant parts were examined and 8 genera of fungi were isolated: *Fusarium*, *Phytophthora*, *Rhizoctonia*, *Phoma*, *Alternaria*, *Sclerotinia*, *Botrytis* and *Ascochyta*. The fungi were isolated in the faba bean plants and there were macroscopically clearly visible symptoms of infection.

Key words: faba bean, mycopopulation

Introduction

Faba bean is botanically known as *Vicia faba* L., with the common name broad bean. It is one of the earliest domesticated food legumes in the world belonging to the family Fabaceae.

It is originally of African-Asian origin, and it is grown all over the world. Its height can be 50-100 cm, grows upright, and there are no climbing vines. The plant is self-fertilized. It is rarely found in wild form in nature, only cultivated species are known.

Faba bean, as a field, annual legume, has been known since ancient times. Faba bean was cultivated in ancient Egypt, Greece, and Rome, as confirmed by numerous records. Except for human nutrition, it was, also, used as a green fertilizer. Today, faba bean plants are used as both garden and fodder crops in many countries of the world.

In Europe, it is cultivated in England, Belgium, Scandinavian countries,

Germany, Russia and Italy (Mišković, 1986). In our country, faba bean is seldom grown, especially as a fodder. Faba bean is a multifaceted fodder crop. The content of nutrients in the grain of the faba bean and in the plant as a whole is high.

Nutrient value of faba bean is high, with about 30 % of protein. Due to its content, faba bean serves as a great concentrated animal feed for the most species of livestock. Also, faba bean grains are used in human nutrition, as a stew, and it has high nutritional value (Vučković, 1999).

In addition, it is one of the best crops for cropping systems due to its ability to establish symbiosis with specific rhizobia bacteria, which results in biological nitrogen fixation which, in turn, reduces the input of fertilizers (Tegegn, 2017). In spite of its importance, the productivity of the crop can be reduced due to several biotic and abiotic factors. Fungal diseases are among the most important biotic factors causing faba bean yield reduction. Among them, chocolate spot and rust which are considered the most important diseases in the Mediterranean region. Other fungal foliar diseases could contribute to the low productivity of the crop, such as Ascochyta blight, Alternaria leaf spot, root rot, white mold (*Sclerotinia* stem rot), downy mildew and Cercospora leaf spot (Salam et al., 2011).

They have a significant impact on the reduction of potential yield of these cultures, but also the quality of the final product, trade of plant material and expansion of legumes into new areas (Porta-Puglia and Aragona, 1997). Vetch and bean anthracnose is caused by the fungus *Ascochyta fabae* Speg. (teleomorph *Didymella fabae* G.J. Jellis & Punith.) (Tivoli et al., 2006). *Uromyces viciae-fabae* (Pers.) J. Schröt. is the causal agent of the rust in faba bean and has a wide range of hosts, species from the genera *Pisum*, *Lathyrus* and *Lens* (Sillero and Rubiales, 2014). Two *Fusarium* species were determined on the cultivated legumes seeds, *F. verticillioides* and *F. proliferatum*, respectively (Miličević et al., 2013).

Similarly, *Erysiphe pisi*, *Botrytis cinerea*, *Rhizoctonia solani*, *Cercospora medicaginis*, *Pseudopeziza medicaginis*, *Sclerotinia trifoliorum*, *Stemphylium botryosum*, *Verticillium albo-atrum*, *Aphanomyces euteiches* as well as *Phytium*, *Leptosphaerulina*, *Phoma*, *Phytophthora* and *Alternaria* species are significant disease agents in faba bean, spread in all production areas (Villegas-Fernández and Rubiales, 2011; Salam et al., 2011). In case of severe infections a serious damage to faba bean seedlings could be caused. Fungal diseases are among the major biotic threats that lower the yield of the crop. Chocolate spot (*Botrytis fabae*), rust (*Uromyces vicia faba*) and Ascochyta blight (*Ascochyta fabae*) are the most frequently prevailing fungal diseases of faba bean which can cause considerable loss of yield (Tegegn, 2017).

Considering the importance of faba bean as a fodder crop in Serbia, the aim of this study was to identify phytopathogenic fungi as casual agents of diseases in faba bean for a clearer perception of problems (the extinction of plants, reducing

yields, deterioration of the quality of feed and other) arising as a result of the presence those fungi.

Materials and Methods

For the mycopopulations study, samples were collected from the experimental seeds genotypes of faba bean (*Vicia faba* L.) originating from Serbia, from the Rasina region (Gaglovo 1, Koševi), Zaječar region (Resnik, Rujevac, Mužinac), Nišava region (Praskovče) and Pomoravlje region (Jagodina 1, Jagodina 2, Rekovac1, Rekovac 2).

The samples were collected between March and June 2016-2017 at the location of the Institute for Forage Crops in Globoder. Plant parts were carefully washed under running water. After washing, the parts of stem and roots were cut to pieces of 0.5-1 cm in size. Prepared samples of roots and stems were disinfected with 96% ethanol for 10 seconds and with 1% sodium hypochlorite (NaOCl) for 1 minute and then washed three times in sterile distilled water. They were then dried on sterile filter paper and placed on potato dextrose agar medium (PDA) with streptomycin. Five pieces of the plant parts (roots and stem) were placed each in Petri dishes in four replications. They were kept in a thermostat at 25°C in 12 h light/12 h night regime. The observations were performed every 3 days, and the majority of mycelium samples were developed up to 14 days. Developed mycelia were screened to a new PDA substrate and, after an initial grow, the peak part of the mycelium was reseeded on PDA again.

Microscopic examination was performed using microscopes Olympus CX31 (Europa SE & Co. KG., Hamburg, Germany). Morphological identification of fungi to the genus was carried out using a standard key. Calculated by the frequency of isolation in % according to the formula Vrandečić *et al.* (2011):

$$(\%) \text{ Isolation frequency} = \frac{\text{Number of segments containing the fungal species}}{\text{Total number of segments used in the isolation}} \times 100$$

Results and Discussion

In the study of mycopopulations of of faba bean genotypes, total of 400 plant parts were analyzed. Fungi were isolated on all plants from faba bean, and there were clear symptoms on stems in the form of spots and necrotic lesions.

Fungi from genera *Alternaria* and *Botrytis* were isolated from these plants. In some plants, black fruiting bodies (pycnidia) were observed on stems, which have been found to belong to the genera *Phoma* and *Ascochyta* (Table 1).

Table 1. The percentage distribution of faba bean diseases in Serbia

Genotypes	Number of samples		Fungi species - stem	(%) Isolation frequency	Fungi species - root	(%) Isolation frequency
	Plant part- stem	Plant part- root				
Resnik-Sokobanja	10	30	<i>Alternaria</i> sp. <i>Ascochyta</i> sp.	70 30	<i>Fusarium</i> sp.	50
Jagodina 1	10	30	<i>Phoma</i> sp. <i>Botrytis</i> sp.	10 50	<i>Fusarium</i> sp.	26.27
Rujevica-Sokobanja	10	30	<i>Alternaria</i> sp. <i>Ascochyta</i> sp.	30 10	<i>Fusarium</i> sp. <i>Sclerotinia</i> sp.	23.33 6.67
Gaglovo-1	10	30	<i>Ascochyta</i> sp.	30	<i>Rhizoctonia</i> sp. <i>Fusarium</i> sp. <i>Sclerotinia</i> sp.	10 16.67 6.67
Jagodina 2	10	30	<i>Phoma</i> sp. <i>Ascochyta</i> sp.	80 10	<i>Rhizoctonia</i> sp. <i>Fusarium</i> sp.	16.67 43.33
Rekovac 1	10	30	<i>Alternaria</i> sp. <i>Ascochyta</i> sp.	20 50	<i>Fusarium</i> sp.	60
Mužinac-Sokobanja	10	30	<i>Alternaria</i> sp. <i>Botrytis</i> sp.	50 10	<i>Fusarium</i> sp.	50
Praskovče	10	30	<i>Alternaria</i> sp. <i>Ascochyta</i> sp.	30 30	<i>Fusarium</i> sp. <i>Sclerotinia</i> sp. <i>Rhizoctonia</i> sp.	16.67 13.33 13.33
Koševi	10	30	<i>Botrytis</i> sp. <i>Ascochyta</i> sp.	50 20	<i>Fusarium</i> sp. <i>Phytophthora</i> sp.	36.67 10
Rekovac 2	10	30	<i>Botrytis</i> sp. <i>Ascochyta</i> sp.	20 40	<i>Sclerotinia</i> sp. <i>Fusarium</i> sp.	26.67 16.67

The symptoms of a light to dark brown necrosis on the root system of the plants were observed, and from these plants fungi of the genera *Sclerotinia*, *Phytophthora* and *Rhizoctonia* were isolated. Discoloration of the conductive tissues of the root system was observed in a large number of plants and from these plants fungi of the genus *Fusarium* was isolated (Table 1).

The results indicate that faba bean is vulnerable to the large number of phytopathogenic fungi that can have a significant impact on reducing its yield and quality. Isolations were conducted in all the faba bean plants with clearly visible symptoms of the disease.

In these studies, there was a difference in the frequency of isolation of certain genera of phytopathogenic fungi in faba bean genotypes originated from different regions of Serbia. It has been observed that in faba bean genotypes that originated from the Zaječar region, fungi of the genera *Alternaria* and *Fusarium* were more frequently isolated. Likewise, genera *Botrytis*, *Ascochyta* and *Phoma* were more often isolated from genotypes originated in the Pomoravlje region. As for the remaining three genera, isolated phytopathogenic fungi were present in all studied faba bean genotypes.

Faba bean is attacked by a number of fungal diseases, among which are chocolate spot (*Botrytis fabae*), *Ascochyta* blight (*Ascochyta fabae*) and rust (*Uromyces vicia faba*) are the major ones. Leaf diseases limit the production of faba beans. In Ethiopia, chocolate spot and rust are the major diseases which can reduce yield by about 61 and 21%, respectively (Tegegn, 2017).

Genera *Fusarium*, *Phytophthora*, *Rhizoctonia*, *Phoma*, *Alternaria*, *Sclerotinia*, *Botrytis* and *Ascochyta* are dominant in annual and perennial legumes worldwide (Tivoli et al., 2006; Villegas-Fernández and Rubiales, 2011; Salam et al., 2011; Sillero and Rubiales, 2014, Vasić et al., 2015, Vasić et al., 2017). While Salam et al. (2011) cited genera *Ascochyta* and *Botrytis*, especially species *Ascochyta fabae* Speg. (teleomorph: *Didymella fabae*) and *Botrytis fabae*, *Botrytis cinerea* as significant pathogens on faba bean in Australia. It is important to mention that the parasites of the genus *Botrytis* overwinter in the form of sclerotia or mycelium into plant residues in the soil (Davidson et al., 2004). Coca-Morante and Mamani-Álvarez (2012) reported that in Bolivia those that most affect of faba beans are caused by the fungi *Alternaria alternata*, *A. tenuis*, *Botrytis cinerea*, *B. fabae*, *Mycosphaerella* sp., *Oidium* sp. and *Uromyces viciae-fabae*. Al-Jaradi et al. (2018) in Oman detected *Fusarium equiseti* on *Phaseolus vulgaris*. In the pathogenicity test, treated *P. vulgaris* showed yellowing symptoms with presence of lesions and root rot on the taproot. *F. equiseti* is one of the causal agents of foot and root rot disease which infects *Phaseolus vulgaris*, *Pisum sativum* and other crops. Al-Jaradi et al. (2018) also isolated *Rhizoctonia solani* from *Vigna unguiculata* in Oman. *Alternaria alternata* resulted in lesions and root rot symptoms on the taproot of *Vicia faba*. *Alternaria* has been detected on different *Vicia faba*, *Pisum sativum* and *Vigna unguiculata*. However, *Alternaria* spp. are usually known to be foliar pathogens, causing various types of spots and blights. This may explain why they were less pathogenic on the roots compared to other fungi (Al-Jaradi et al., 2018).

So, for these reasons, it is recommended to utilize crop rotation of four years, when it comes to the sowing of faba bean and pea (Salam et al., 2011). Salam et al. (2011) also cited *Phoma medicaginis* var. *pinodella* and *Ascochyta pisi* as significant pathogens in pea. *Phytophthora medicaginis* was recorded on

chickpea in Australia and it was also found that this parasite can infect other types of legumes (Salam *et al.*, 2011). *Rhizoctonia solani* Kühn is soil parasite that can cause serious problems in many legumes, especially on faba bean (Assunção *et al.*, 2011). In Canada, 304 faba bean genotypes were tested on the resistance to *R. solani* and only five of them were identified with high resistance (Rashid and Bernier, 1993). In Benghazi, phytopathogenic fungi such as *Botrytis*, *Uromyces*, *Alternaria* and *Ascochyta* can seriously reduce the seed quality and spread the disease to the next season, Tegegn (2017).

According to Tegegn (2017), chocolate spot reduces yield by up to 61%, with the presence of the probability of complete crop failure due to the disease. (Tegegn, 2017) reported that rust can incur a maximum yield loss of up to 21%. So far, the control of these diseases had been attempted through the use of improved cultivars, cultural practices, chemical fungicides and integration of two or more of the above options in Integrated Disease Management (IDM) scheme.

Conclusion

This paper presents the preliminary results of mycopopulations of 10 experimental faba bean genotypes. Faba bean is an important forage crop and its importance as livestock feed is growing within our country. This research is the beginning of a more comprehensive study of phytopathogenic fungi on faba bean. So far, there were no significant researches in this direction in Serbia, so the future researches related to faba bean will go in the direction of selection of genotypes with increased tolerance to fungal diseases.

Mikopopulacija različitih genotipova boba u Srbiji

Tanja Vasić, Snežana Andjelković, Jordan Marković, Sanja Živković, Đorđe Lazarević, Mladen Prijović

Rezime

Bob (*Vicia faba* L.) je jednogodišnja biljka iz porodice mahunarki (*Fabaceae*). Potiče iz umerenog klimatskog pojasa Afrike i Azije. Ima posebno mesto u obezbeđenju kabaste stočne hrane (krme) u zoni umerenog klimata. Pripada visokokvalitetnim proteinskim krmnim biljkama.

Sistematskih istraživanja mikoflore boba u Srbiji do sada nije bilo. U ovome radu iznosimo rezultate preliminarnih istraživanja mikopopulacije 10 različitih genotipova boba.

Ukupno je pregledano 400 biljnih delova sa kojih je izolovano 8 rodova gljiva: *Fusarium*, *Phytophthora*, *Rhizoctonia*, *Phoma*, *Alternaria*, *Sclerotinia*, *Botrytis* i *Ascochyta*. Na biljkama iz kojih su izolovane gljive bili su jasno vidljivi makroskopski simptomi zaraze.

Ključne reči: bob, mikopopulacija

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