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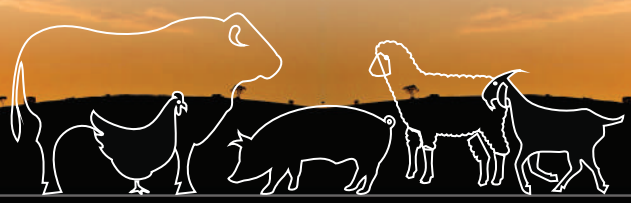
NEW PERSPECTIVES AND CHALLENGES OF SUSTAINABLE LIVESTOCK PRODUCTION



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New Perspectives and Challenges of Sustainable Livestock Production
October 7 – 9, 2015

TREND AND CURRENT SITUATION IN ANIMAL HUSBANDRY OF SERBIA

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Invited paper

Abstract: Despite the very favorable natural conditions, animal breeding in Serbia are still looking for a way to stabilize production. In the last decade of the 20th century, livestock in Serbia almost halved. It reduced the number of animals and total production of all types of meat. The small farms were dominant in Serbian agriculture with an average area between 3 and 4 ha. At the end of 2014, compared to 2006 the number of animals in Serbia reduced by 16.81% in cattle, 19.09% in pigs, and 26.75% in goats. Compared to 2006, we see that the fund sheep in 2014 increased by 10.98%. production of cow's milk at the end of 2014 decreased compared to 2006 by 5.98%. Unlike cattle, in sheep is an evident trend of increase in milk production by as much as 25% in the period. Goat milk from 2006 to 2014, varied and we can say that in 2014 we produced less milk 7.31%, however, shows that the quantity of milk per animal increased, as a result of selection and better growing conditions. The amount of wool decreased by 2,52% in 2014, however, due to the low interest for wool farmers is not recorded, so that the statistical indicators are not entirely realistic. Egg production increased by 21.7%, in line with the growing number of of poultry. Serbia produces 452.000 tons of meat. From that number 73,000 tons of beef, 27,000 tons of sheep meat, 258,000 tons of pork, and 94,000 tons of poultry meat. Total meat production in our country has a tendency of stagnation or slight decline.

Keywords: animal breeding trend, cattle, sheep, pig, poltry, meat, milk, wool, egg

Introduction

In the Republic of Serbia, According to the Statistical Office of country there are 631.122 farms constituting of 2.567 holdings of legal entities and

entrepreneurs and 628.555 family farms/agricultural holdings. The agricultural area covers around 5.100.000 ha, out of which 83% - that is around 4.250.000 ha - is cultivable. In Serbia, farmers comprised 17.3% of the total population. The distributions of the population working in farms in Serbia were as follows: animal production 43%, crop production 42%, production of vine and grapevine 12%, and other crops 3% (*Bogdanovic et al., 2005; Petrovic et al., 2012; 2013*. Republic of Serbia has significant natural resources (agricultural land, air, water, etc.) and very significant capacity and resources (agricultural population, livestock population, manufacturing and processing facilities and techniques, developed educational scientific activities, etc.). The current level of livestock production in Serbia does not provide cost-effectiveness, therefore it is necessary to work more efficiently and to change the same organization to enhance capacity building in qualitative and quantitative term (*Aleksić et al., 2007; 2009; Petrovic et al, 2012).*

Despite the very favorable natural conditions, cattle breeding in Serbia are still looking for a way to stabilize production. In the last decade of the 20th century, livestock in Serbia almost halved. It reduced the number of animals and total production of all types of meat. The small farms were dominant in Serbian agriculture with an average area between 3 and 4 ha. Process of increasing the efficiency of livestock production may also contribute to the linking of stage of production and processing in a single production cycle, which requires forming of the association of producers.

Bearing in mind that Serbia has potential livestock production, special attention should be given and its share in the total increase agriculture as is the case in developed countries. The aim of this study was to assess the trend intensity changes and the current situation in livestock production.

Table 1. The number of heads of all species of domestic animals (000 heads)

Species	2006	2007	2008	2009	2010	2011	2012	2013	2014
Cattle	1106	1087	1057	1002	938	937	921	913	920
Swine	3999	3832	3594	3631	3489	3287	3139	3144	3236
Sheep	1556	1606	1605	1504	1475	1460	1635	1616	1748
Goat	299	275	284	263	237	239	232	225	219
Poultry	16595	16422	17188	22821	20156	19103	18234	17860	17167

From the above table we can see that at the end of 2014, compared to 2006 the number of animals in Serbia reduced by 16.81% in cattle, 19.09% in pigs, and 26.75% in goats. It can view that in recent years in cattle and pigs led to a small increase in the number of animals, which is a good sign. Looking at the same table, we can notice that the number of sheep on the rise. Compared to 2006, we see that the fund sheep in 2014 increased by 10.98%. This increase began from 2012 and the measures are the result of governmental stimulation. In poultry, production has also registered an increase of 3.33%. Changes in the breed structure in cattle population raised in Serbia over the past decade have been intense. Breeds that are more intensive have suppressed the extensive breeds and breeds with poor production, so that they had numerically reduced. Now Simmental and Domestic Spotted in Simmental type make up to about 75% of total population, the group of Black-and-White and Red-and-White Holstein- Friesian cattle around 20%, while the primitive races and crosses make up about 5% of the total number of cattle in the Republic of Serbia *(Petrovic et al.,2013).*

In terms of breed structure various strains of Pramenka make up about half of the total number of sheep, various types of crosses - about 25%, Tsigai breed about 5% and about 20% imported pure breeds used as improvement breeds. In the territory of the Republic of Serbia, the following sheep strains reared are: Sjenica, Svrljig, Pirot, Lipska, Krivovir etc., Tsigai breed sheep, various more or less well-established crosses of different strains of Pramenka sheep and Merino breed. For improvement of sheep breeds in Serbia Merinolandshaf sheep flocks are reared (Wurttemberg breed) as a general improver breed of domestic pramenka strains, Ile de France and Suffolk, as domestic sheep breed improvers to increase the yield and quality of meat *(Petrovic, 2007., Petrovic et al.,2013)* .

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

The poultry are represented light and heavy hybrids from imports, and only a small number of poultry belonging to indigenous populations.

Table 2. Milk production (million liters), wool (tons) and eggs (million pcs.) per specie

Species	2006	2007	2008	2009	2010	2011	2012	2013	2014
Cow's milk	1587	1571	1561	1505	1485	1462	1465	1451	1492
Sheep milk	15	14	14	10	10	11	12	18	20
Goat milk	41	36	36	28	27	29	33	34	38
Wool	1941	1870	1726	1711	1705	1760	1794	1755	1892
Eggs	1941	1870	1726	1711	1705	1760	1794	1755	1892

Table 2 shows that the production of cow's milk at the end of 2014 decreased compared to 2006 by 5.98%.The reason for this is reduced the number of cows. Unlike cattle, in sheep is an evident trend of increase in milk production by as much as 25% in the period. Here is also the main reason for changes in the number of animals, namely the increased number of sheep. When the amount of Goat milk from 2006 to 2014, varied and we can say that in 2014 we produced less milk 7.31%, however, shows that the quantity of milk per animal increased, as a result of selection and better growing conditions. The amount of wool decreased by 2,52% in 2014, however, due to the low interest for wool farmers is not recorded, so that the statistical indicators are not entirely realistic. Egg production decreased by 2.52%, in line with the growing number of of poultry, but it must be emphasized effect of favorable breeding conditions.

Table 3. Meat production (thousand tons)

Species	2006	2007	2008	2009	2010	2011	2012	2013	2014
Beef	83	95	99	100	96	81	82	70	73
Mutton	20	20	23	24	23	24	22	30	27
Pork	255	289	266	252	269	271	252	249	258
Chicken	70	75	76	80	84	103	94	92	94
Total	428	-	-	-	-	-	-	-	452

From table 3 we can observe that Serbia produces 452.000 tons of meat. From that number 73,000 tons of beef, 27,000 tons of sheep meat, 258,000 tons of pork, and 94,000 tons of poultry meat. Total meat production in our country has a tendency of stagnation or slight decline. Table 3 also showed that dominated pork

meat production with the participation of 57.07% of total production, beef takes 16.15 %, poultry 20.79 % and sheep 5.97 %.

The total average annual production of beef for the past years in Serbia was about 86,000 tones, with a tendency of decline, especially in recent years, and the latest data show that it is about 73,000 tons. Exports of cattle for slaughter and beef dropped drastically to meet less than 20% of its quota to the EU (8700tonnes). Production of this type of meat in the Republic of Serbia has varied from 156,000 in year 1985 to 73,000 t in 2014. This quantity is sufficient meat for the domestic market, as the average consumption per capita is about 12 kg. Lower production of beef compared to Serbia have some Scandinavian countries, Portugal, Bulgaria, Greece, etc. *(Petrovic et al., 2013*).

Conclusion

Despite the great importance and favorable natural conditions in livestock production on the basis of available data, it could estimate as unsatisfactory. In recent years, the number of heads of important species of domestic animals is continuously decreasing. Serbia has granted a candidate status in the European Union (EU). This means that livestock production should prepare for competition on unique developed market, without any state trade barriers. Serbia's membership in international organizations, liberalization of trade in livestock products, low possibility for import protection reduced levels of domestic support, elimination of export subsidies, the great competition on the international market, are still additional challenges faced or will face Serbia livestock breeding. Based on these circumstances, it is necessary to build the principles for the efficient development of livestock production, which may provide greater export opportunities and contributes to the growth of agriculture and national income. The most important partners of Agriculture and Food Industry of Serbia may, in addition to the EU and CEFTA countries, the Russian Federation and the market of the Middle East.

Trend i trenutna situacija u stočarstvu Srbije

M.P. Petrovic, M.M. Petrovic, V. Pantelic, V. Caro Petrovic, D. Ruzic Muslic , M.I. Selionova, Z. Ilic, N. Maksimovic

Rezime

Uprkos veoma povoljnim prirodnim uslovima, stočarstvo u Srbiji i dalje traži način da stabilizuje proizvodnju. U poslednjoj deceniji 20. veka, stočsrstvo u

Srbiji je skoro prepolovljeno. Smanjen je broj životinja i ukupna proizvodnje svih vrsta mesa. Krajem 2014. godine, u odnosu na 2006. godinu broj životinja u Srbiji smanjen za 16,81% kod goveda, svinja za 19.09% i 26.75% koza. U odnosu na 2006. godinu, fond ovaca u 2014. povećan za 10.98%. Proizvodnja kravljeg mleka na kraju 2014. godine smanjen u odnosu na 2006. godinu za 5,98%. Za razliku od goveda, kod ovaca je evidentan trend povećanja proizvodnje mleka od čak 25% u istom periodu. Kozje mleko od 2006. do 2014. godine, varira, pa je u 2014. godini proizvedeno manje za 7.31%, međutim, pokazuje se da je količina mleka po životinji porasla, kao rezultat selekcije i boljih uslova gajenja. Prinos vune smanjen je za 2,52% u 2014. godini. Proizvodnja jaja porasla je za 21,7%, u skladu sa rastućim brojem živine. Srbija proizvodi 452.000 tona mesa. Od tog broja 73.000 tona junećeg mesa, 27.000 tona ovčijeg mesa, 258.000 tona svinjskog mesa, i 94.000 tona živinskog mesa. Ukupna proizvodnja mesa u našoj zemlji ima tendenciju stagnacije ili blagog pada.

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TRENDS FOR THE MANAGEMENT IN LIVESTOCK FARMING

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Invited paper

Abstract: In near future global production of food with farm animals increases. Three reasons are responsible:

- Increase the population on earth from 6 to 8 billion in 2013 and more than 10 billion in 2070.
- Reducing of the available area and all resources per person,
- Higher living standard for people is associated with higher consumption of food of animal origin.

Especially in Europe with higher metabolism in animals, with higher performances of them "Animal welfare" becomes the focus in public discussion.
Global three systems for dairy production emerge.

- High Input – High Output. The aim is highest milk yield more than 10.000 kg/ cow and year by using of high technical equipment and high level of breeding and feeding.
- Low Input – Medium Output: Minimizing costs to realize medium milk yield by about 7.500 kg /cow and year.
- Milk production based on pasture exclusively. Costs are very low.

In Germany the number of dairy farms decrease and the number of cows per farm increase more and more. The proportion of automatic milking systems (robots) rises strongly. Pig production rises, especially finishing farms. Piglet production is based on group farrowing system. Zootechnical methods for reproduction management are special and refined, so that biotechnical methods become less necessary. More than 12 Mio piglets are imported to Germany annually from EU-countries. The increase in reproduction performance in sows will continue to be in next time. Various methods to improve the rearing performance of sows are applied. In Denmark natural nurses are used mostly, in Germany cups for additional milk supply are used successfully.

Key words: animal production, development trends, animal welfare

Introduction

Current and future development of global population is one of the most important challenge for agriculture. Thus further themes connect, among other things like energy, industry, natural resources, environmental protection and other ones. About 800 Mio people are undernourished. About 75% of world livestock in tropics and subtropics produce less than 50% of edible protein of animal origin.

Compared with other sectors of economy and industry, agriculture has the highest growth. Agriculture is livelihood for 1.3 billion people. Livestock production accounts for 40% of agricultural production.

Generally the global importance of farm animal production rises. This situation is justified by three aspects:

- Increase the population on earth from about 6 to 8 billion in 1930 and more than 10 billion in 2070.
- Reducing of the available area and all resources per person, for example water, energy sources, minerals like phosphorus and other ones.
- Higher living standard and higher incomes for people are associated with higher consumption of food of animal origin - "Welfare indicator" with high hedonic value.

The global meat and milk production will increase by about 100% between 2000 and 2050 (tab. 1).

Table 1. Development of global meat and milk production

Sector / year	2000	2050
Meat production	229 Mio t	465 Mio t
Milk production	580 Mio t	1.04 Mrd.t

Intensification is necessary. But this is limited. A replacement low powerful animals by high performance animals would be interesting, but not realizable generally. It affects people, families with their characteristics in climatically, culturally different areas with long traditions, religions and worldviews. It is known that the level of milk production in cows is in direct relations with the demand and expense for energy and methane excretion (fig. 1).

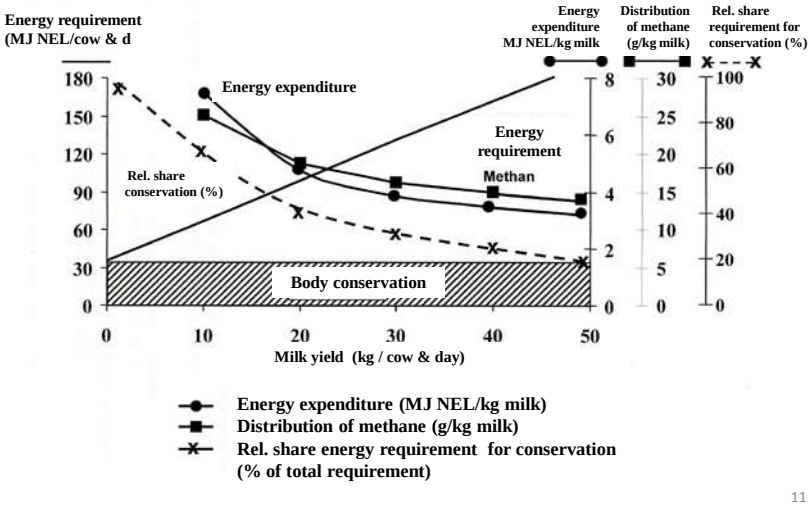

Figure 1. Influence of milk yield on energy requirements, energy expenditure and distribution of methane (Flachowsky, 2012)

Of course in front of this background for agriculture in most of European countries other specific tasks drive. Environmentally sound and sustainable intensification is in focus.

"The efficiency of systems for research and knowledge transfer in agriculture and ecology is the increasingly decisive location factors in situation with scarce agricultural land and scarce water resources.

Therefore it is in terms of a long-term global food policy also an ethical obligation of the European countries do not reduce their capacity to produce knowledge in agricultural sector and systems for knowledge transfer but provide long-term for the World Food" (*Braun, 2001*).

With higher metabolism in animals, with higher performances of cows and sows "Animal Welfare" becomes the focus in public discussion. *Hoy (2102)* divides the development into three phases:

Phase I: Phase is finished. In laws and regulations (Tierschutz-Nutztierhaltungsverordnung) basic requirements are prescribed, for example tying of sow in box.

Phase II: This phase is in present time. There is an intensive public discussion about surgical interventions in animals, like grind teeth in newborn piglets, castration of male piglets, tail docking in newborn piglets or dehorning of calves.

Phase III: This phase has begun. There is the public discussion about following question: "Are high performances in farm animals to animal welfare compatible generally?"

For a description of trends for management in livestock farming examples of dairy farming and piglet production from Germany are given.

Milk production

The biggest milk producers in the world are European Union (annually 161 Mio tons), India (annually 145 Mio tons) and USA (annually 94 Mio tons). Globally, the following systems for dairy production emerge (SWALVE, 1999):

a) High Input / High Output: It includes intense management with particularly intense feeding. The aim is highest milk yield, more than 10.000kg/cow/year. Often it needs higher frequency for milking.
b) Low Input / Medium Output: It focuses minimizing the costs for building and feed. Expected milk yield is about 7.500 kg /cow/year. More than 50% of basic forage is on pasture (*Weigel et al. 1999).*
c) Milk production based on pasture: Production exclusively based on grazing (e.g. New Zealand). Costs are very low. Performance optimizing mainly on number of animals /ha (stocking density).

Generally for system a) and b) the same type of cow may be used as no substantial genotype by environmental interaction between these two systems can be detected. For system c) a different type of cattle is necessary.

A further increase of dairy production by means of breeding is possible (*Swalve, 1999*). A selection plateau has not yet been reached. The current growth of knowledge of genome and molecular genetics is high and the estimation of breeding value by genomic selection will be more effective, especially for functional traits.

A higher metabolic rate requires higher demands on the environment. Irrespective of this, changes occur in the structures of German dairy husbandry (tab. 2).

The number of cows per farm increase continuously. In present time in East Germany 16.5% of dairy farms have more than 500 cows. In West Germany are only 0.2% farms of this size. Following in East Germany 48.2% of cows are in farms with more than 500 animals, in West Germany only 1.4%.

Table 2. Development of the structure of recorded dairy herds in Germany (ADR)

		2000	2005	2010	2014
Cows/farm	Germany	44,1	37,7	45,7	56,2
	East	192,4	171,7	156,1	182,2
	West	30,1	31,8	39,6	48,7
Milk yield /cow & year (German Holstein)		6.110	8.282	8.766	8.994

The investments indicate the future development of management systems in dairy industry. In following years about 15.2% of dairy farmers (100 – 200 cows/farm) want to buy new milking systems. A lot of them want to invest in automatic milking systems (robots). About 40% of these farmers are interested for robots than in traditional equipment. Until now in farms with more than 200 cows traditional milking systems are preferred. However, it is a growing interest in automatic milking system recognizable. These systems are based mostly on multiple robots (about 50 cows /robot). Since 2 years in Germany first Automatic Milking systems Rotation (AMR) are used. The main reason of this development is a higher productivity with reduction of staff and higher level of Animal Welfare.

Pig production

In German pig production a strong progressive development is observed. In January to June of 2015 in Germany 30 Mio were slaughtered, so many like never before. Especially pig finishing has a very progressive development in Germany. More than 12 Mio piglets are imported to Germany annually, especially from Denmark and Netherland. In some regions of Germany, like North-West and South Germany, the stocking density of animals reaches the upper limit now. For future following facts are important:

- Farms and stocks enlarge,
- Number of pig farms will be reduced,
- In large stocks with colleagues a specialization effect is effective and leads positive results. Colleagues are working only in stable with the same kind of animals. That is good for high quality of work.
- Performance in reproduction and growth of animals increase. This situation needs more modern stables with good housing and feeding conditions.
- Generally, this complex process is combined with continuous improvement of animal welfare.

The successfully tested production systems in piglet production are applied nationwide (fig. 2). These system include group farrowing and weaning in 7 day-rhythm for bigger farms and in 21-day-rhythm for farms with midsize stocks.

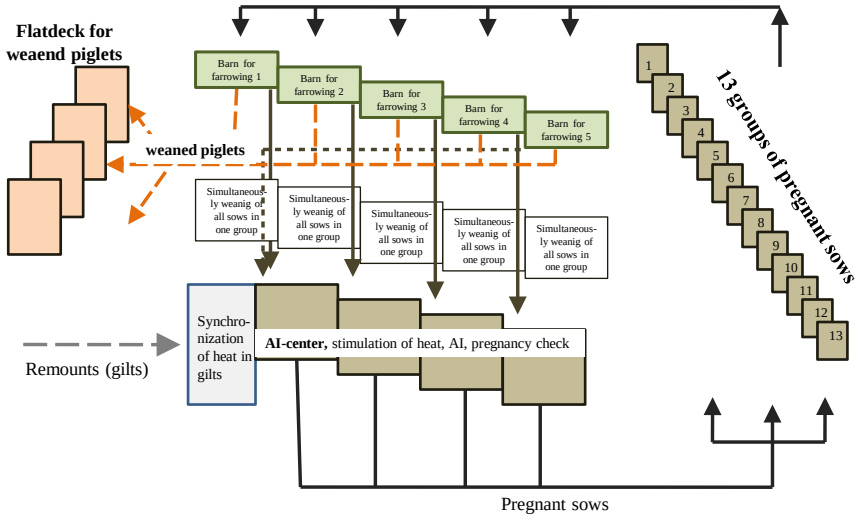

Figure 2. Scheme for piglet production with group farrowing system in 7-day-rhythm (22 groups of sows, 5 pigsties for farrowing)

In management programs for piglet production intensive zootechnical stimulation of puberty and heat in gilts and sows is one of the most important facts for high reproduction performance. The methods are very special and refined. They differ from farm to farm. Often hormonal stimulation of estrus and ovulation is no longer necessary generally. Biotechnical methods are often used temporary, depend on season or parity of sows.

In the past 6 years the number of weaned piglets per sow and year has been increased in the average 1 piglet per year. This strong progress is the result of breeding, nutrition and husband-dry management and of better animal health (fig. 3).

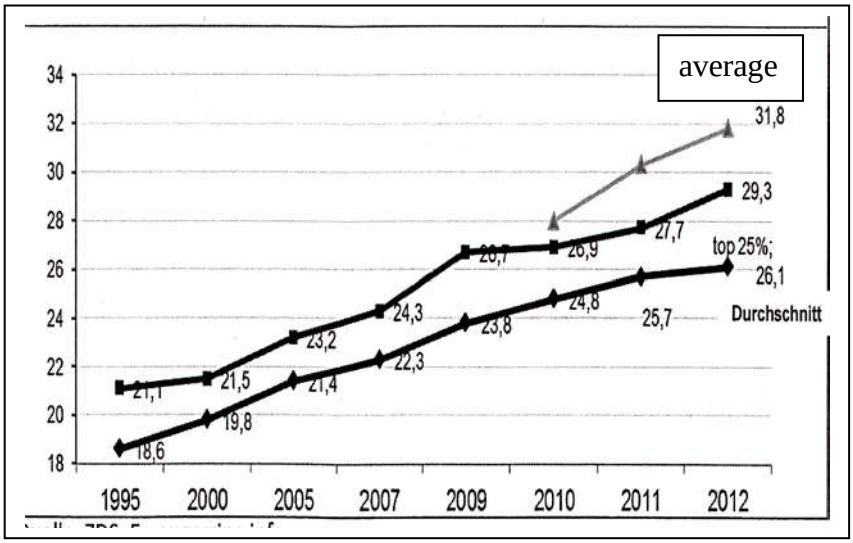

Figure 3. Development of reproduction performance in sows and number of weaned piglets per sow and year (ZDS 2012)

This process is not yet completed. *Hortmann-Scholten (2014)* warns against limiting reproduction performance. The breeding success in reproduction performance is necessary for positive economical situation in farms. If there is a better litter size, economies of scale is acting. If there are 32 sold piglets per sow and year, author realized lower costs by 20% /piglet compared only 24 sold piglets. Breeding companies have created good genetic conditions for high fertility in sows.

In present time breeding organizations and companies are more active for better vitality of piglets. That is important for sustainability in farms and in animals. One of the aims of activities are sows with high quality in udder and high level in uniform litters with stable reproduction performance. Generally, with increased litter size the input of man power, technical systems, feed quality, financial expenses a.s.e., is increasing too. That is necessary to keep piglet losses in low level in future. That is an important ethically justified. Different management measures are used for these aim (Tab. 3).

Table 3. Management measures in piglet production

Before and during parturition	**After parturition and others:**
- Synchronization of parturitions in group of sows after timed AI	- Litter balance /cross forstering
- Birth care	- Supplementation of additional milk
- Split Nursing	- In time and enough feeding of piglets (prestarter for suckling piglets)
- Desinfection of udder	
- Replacement of colostrum	- System of nurses: natural nurses artificial nurses
	- Housing system in farrowing barn
	- Box for early weaned piglets
- Climate	- Climate
- Hygiene	- Hygiene

Everywhere a lot of farm-special activities to reduce piglet losses can observed. One important goal is to keep the costs. No all management measures are sustainable enough. Not all management measures are tested by scientific researches. Often you can find special experiences of colleagues for animal welfare. Often effectiveness and success vary between farms. The perceived success is not equal to a measured success generally. The pig production has moved increasingly into the focus of public interest in recent years. The proper management of large litters with the aim to reduce the suckling piglet losses, to improve the breeding results is one of the aspects in the context of cross-industry initiative animal welfare. The interest of farmers to actively participate here is great.

In different regions of Germany you can observe a sustained progress in reproduction performance but with constant animal losses (fig. 4).

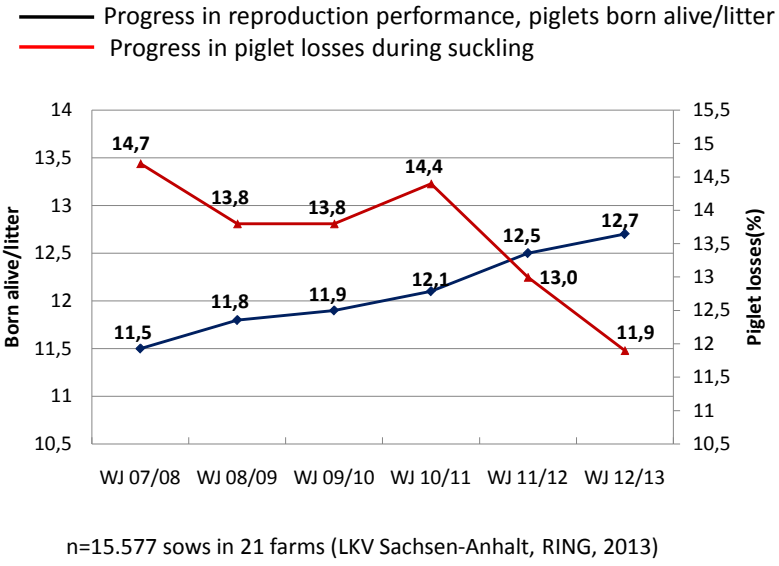

Figure 4. Development in litter size and piglet losses during suckling

When litter size increase continuously, the quality of sow, quality of their udder must come into focus more. Often more piglets were born than teats in udder of sow. Own investigations show, a good help is the application of usage of cups for additional milk supply for suckling piglets. Table 4 shows the positive results in piglet rearing of sows depend on milk-cups.

One more piglet could weaned after 4 week suckling, when milk cups were used for additional milk supply for piglets. The effect was in week one and two of suckling. Animal losses in this time was reduced significantly.

Table 4. Rearing performance in lactating sows in piglet depend on milk-cups

	Experimental group	Control group
Number of litters	43 (40)	42
Age (no.of litter in sows)	2,7 ± 2,0	2,7 ± 1,9
Born total	14,6 ± 3,7	15,7 ± 3,3
Born alive	14,4 ± 3,5	15,3 ± 3,3
Weaned piglets	13,5 [a] ± 1,5	12,5 [b] ± 1,5
Body weight of weaned piglet	6,76 ± 1,5	6,7 ± 1,5

a,b …significant ($p<0,05$)

Conclusion

More ground and resources are not available on earth. The utilization of production factors are necessary. Increasing agricultural production must be realized through more intensity.

Change process in agriculture is proceeding rapidly. The number of livestock farms will decrease and the number of animals per farm will increase generally.

The performance trends of cattle and sows subject to a process by adjusting the breeding goals with increased focus on health and stability. It means functional traits.

Animal welfare regulations are based on the resources made available to the animal, for cattle and pigs.

In milk production different systems are used: intensive methods and extensive methods. It depends on available of pasture and economic framework.

German dairy farms realize more intensity by modern equipment for milking systems. The tasks of the farmer change towards animal Observation and system controller.

In pig production known production systems (group farrowing) are applied. Biotechnical control of reproduction get a limited importance.

Reproduction performance is increased. A sow has to wean 26 – 30 salable piglets/year. In piglet production numerous activities to improve piglet rearing can be observed.

Trendovi u upravljanju proizvodnjom u stočarstvu

M. Waehner

Rezime

U bliskoj budućnosti se očekuje povećanje svetske proizvodnje hrane od domaćih životinja. Tri razloga su odgovorna:

- Povećanje broja stanovnika sa 6 na 8 milijardi u 2013. godini, a više od 10 milijardi do 2070.
- Smanjenje raspoloživog prostora i svih resursa po osobi

- Viši životni standard za ljude je povezan sa većom potrošnjom hrane životinjskog porekla.

Posebno u Evropi sa bržim metabolizmom životinja, sa očekivanim višim proizvodnim performansama od njih, "Dobrobit životinja" postaje fokus za javnu raspravu.

Globalno postoje tri sistema u proizvodnji mleka:

- Visoki ulaz - visoki izlaz: cilj je najveći prinos mleka više od 10.000 kg/kravi i godini pomoću visoke tehničke opreme i visokog nivoa uzgoja i ishrane
- Niski ulaz - srednji izlaz: minimiziranje troškova za realizaciju srednjeg prinosa mleka od oko 7.500 kg/kravi i godini.
- Proizvodnja mleka na osnovu pašnjaka isključivo. Troškovi su veoma niski.

U Nemačkoj se broj farmi za proizvodnju mleka smanjuje i broj krava po farmi povećava sve više i više. Procenat automatskih sistema za mužu (roboti) pokazuje izraziti porast.

Proizvodnja svinja je u porastu, posebno farmi za tov. Proizvodnja prasadi se bazira na sistemu grupnih prašenja. Zootehničke metode za upravljanje reprodukcijom su specifične i precizno definisane, tako da biotehničke metode postaju sve manje potrebne. Više od 12 miliona prasadi se uveze u Nemačku godišnje iz zemalja EU. Poboljšanje reproduktivnih performansi krmača će i dalje biti prisutno u narednom periodu. Razne metode se primenjuju za poboljšanje proizvodnih performansi u odgoju krmača.

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THE IMPACT OF IMPORT OF BREEDING SIMMENTAL COWS ON IMPROVEMENT OF PRODUCTION PERFORMANCE IN DOMESTIC POPULATION

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Invited paper

Abstract: The aim of this study was to obtain relevant data pertaining to the basic indicators of milk and fertility Simmental cows, in the production conditions on individual farms, using appropriate modern methodologies, and to determine the effect of import of breeding cows on implementation of the breeding programs and improvement of production performance in domestic population on the territory of Šumadija district. Imported animals have realized in all three lactations higher milk production (449.08 kg, 568.52 kg and 488.73 kg), milk fat (19.87 kg, 22.66 kg and 18.52 kg) and 4% fat corrected milk (477.64 kg, 567.26 kg and 473.36 kg), in the first, second and third lactations, respectively. Domestic cows had lower milk fat content in the first lactation by 0.05%, but higher in the second and third by 0.01%. Domestic cows first calved earlier by 79.13 days, the second time by 89.51, and the third time by 88.62 days, and all differences were statistically highly relevant. They also showed statistically significant differences ($p < 0.01$) in the duration of service period where domestic cows had shorter service periods by 10.19 (II lactation) to 15.94 days (III lactation). Based on the results obtained in this study it can be concluded that the imports of high-quality heifers of Simmental breed from Germany significantly influenced the improvement of milk and fertility performance of domestic cow population in the Šumadija district.

Key words: milk performance, fertility, Simmental breed.

Introduction

In regard to the breed structure of cattle in Serbia, it is estimated that the Simmental breed makes up about 80%, a group of Black and White Holstein-Friesian cattle, about 10%, while the indigenous breeds and crossbreds make up about 10% of the total number of cattle. Simmental breed is primarily present in Central Serbia with 115.000 breeding animals, while the number in Vojvodina is much smaller - about 6.000 registrated animals. A similar situation is in the region of Sumadija, where over 90% of cows belong to Simmental breed. The main reason for the above mentioned share of Simmental cattle lies primarily in very good acclimatization abilities, and the ability to adapt to different rearing conditions.

In the last two decades there has been a decline in the number of heads of Simmental breed in Serbia, but also the increase the number of registered cows, and the trend is still present. In order to increase the number and quality of breeding animals, to improve the genetic composition and increase milk production in the Sumadija district, 150 heifers of Simmental breed were imported from Germany.

Perković et al. (2003) have examined the influence of Montbeliard bulls to improve milk production traits and meat production in domestic spotted cattle. Daughters of Montbeliard bulls, compared with daughters of bulls domestic spotted breed, had by 299 kg more milk and 0.54% less fat.

Comparative examination of results achieved in 2 groups of daughters F1 generation, *Kučević et al. (2005)* have concluded that primiparous animals tested in Germany achieved a significantly greater yield of milk, milk fat and fat content (1.057 kg, 41 kg, 0.22%, respectively).

Results of comparative tests of first calving heifers of domestic Simmental and Austrian provenience in the same growing conditions (*Medić et al. 2006)* showed that in imported heads realized higher production of milk by significant 1.171 kg and 0.49% fat.

Examining the production potential of Simmental heifers calved in the period 2007-2010, on individual sector in Serbia, *Nikšić et al. (2011)* have e3stablished their average milk yield of 4.348 kg of milk with 3.93% milk fat and a yield of 171.1 kg milk fat.

Analyzing the phenotypic variability of Simmental bull dams *Pantelić et al. (2013)* have determined milk production of 5754.49kg, with 3.98% and 230.24 kg milk fat or 5755.47kg 4% FCM.

Problems with fertility have become one of the most expensive factors in dairy cattle. Selection on maternal fertility is becoming increasingly important, and requires its greater involvement in the overall breeding value. If there are serious

problems in reproduction, in addition to direct consequences on the milk production and milk fat, difficulties arise in the normal implementation of the renewel of the herd, which is reflected in the economy of production *(Pantelić et al. 2009).*

Material and methods

During the last ten years, a significant number of pregnant heifers, mainly from Germany and Austria have been imported to Serbia. Majority< of imported heifers are Simmental breed followed by the Holstein Friesian breed, while other breeds were imported in much smaller numbers.

Investigation of differences in milk production of domestic and imported cattle and contribution of import of as pregnant heifers as well as frozen bull semen, to the improvement of breed structure and milk production was tested on a total of 303 cows in first three lactations. Heifers and cows were reared on numerous individual farms, but we can say generally in very similar conditions of keeping and feeding. Cows were kept in stalls with tied system, on long and medium places for lying down with straw bedding. Nutrition was based on hay and alfalfa haylage, rarely grass, corn silage and principally mixture of concentrate. Milking was done with machines, mostly into cans, and milk was stored in lacto-freezes until delivery. Control of productivity was done according to the principles of AT4 productivity control by breeding organizations. The following milk production traits in the first three standard lactation were studied:

- Milk yield, kg;
- Milk fat content, %;
- Milk fat yield, kg
- Yield of 4% fat corrected milk, kg.

In addition to milk production traits, for each cow age at calving and the service period in each lactation were determined.

Average values and variability of all studied traits was determined using standard statistical methods, and the significance of the impact of order of lactation, heifers origin and provenience of their bull-sires by applying the *t* and *F* test.

Results and Discussion

Milk production in Serbia is still carried out on relatively small farms, often with less than 10 cows, but it is not negligible, nor in terms of the total quantity of milk produced, nor in terms of the number of people engaged in it. In

conditions of social transition, milk production, even for farmers with smaller number of cows, is an important source of livelihood. By importing pregnant heifers farmers want primarily to increase the strength of their herds, and improve production and genetic potential on their farms, because the Serbian market for quality heifers is still underdeveloped.

Table 1. Average values and variability of investigated milk production traits of domestic cattle (n=245)

Indicator	$\bar{X}$	SD	CV	Min.	Max.
First lactation					
Milk yield, kg	4385.97	366.30	8.35	3166	5831
Content of milk fat, %	3.91	0.08	2.00	3.37	4.18
Yield of milk fat, kg	171.65	14.93	8.70	116.83	233.24
Yield of 4%FCM, kg	4329.10	367.88	8.50	3019	5831
Second lactation					
Milk yield, kg	4970.89	378.80	7.62	3836	6021
Content of milk fat, %	3.93	0.06	1.65	3.71	4.13
Yield of milk fat, kg	195.51	15.14	7.74	148.07	237.08
Yield of 4%FCM, kg	4921.03	376.55	7.65	3755	5931
Third lactation					
Milk yield, kg	5351.98	398.45	7.44	4129	6690
Content of milk fat, %	3.95	0.07	1.83	3.35	4.14
Yield of milk fat, kg	211.35	16.06	7.60	165.16	267.60
Yield of 4%FCM, kg	5311.12	397.43	7.48	4129	6690

Cows from domestic rearing/breeding, as primiparous, achieved in standard lactation yield of 4385.97 kg of milk with 3.91% fat, and milk fat yield was 171.65 kg, and the yield of 4% fat corrected milk 4329.10 kg. The values of investigated indicator of milk performance, respectively, in the second standard lactation were: 4970.89 kg; 3.93%; 195.51 and 4921.03 kg. In the third lactation cows achieved the highest yield of milk (5351,98kg), milk fat (211.35 kg) and 4% fat corrected milk, as well as the highest milk fat content (3.95%).

Lower production results for the first calving Simmental cows reared in our conditions, were recorded in the research of *Đurđević et al. (1994), Miščević et al.(1995)* and *Petrović M. (2000),* and approximately the same *Perišić (1998),* and *Gutić et al. (2002)*.

Results of milk production in the first three standard lactations of imported heifers are shown in Table 2.

Table 2. Average values and variability of investigated milk production traits of imported cattle *(n=58)*

Indicator	$\bar{X}$	SD	CV	Min.	Max.
First lactation					
Milk yield, kg	4835.05	497.68	10.29	3780	5844
Content of milk fat, %	3.96	0.04	1.06	3.89	4.07
Yield of milk fat, kg	191.51	19.83	10.35	151.20	233.60
Yield of 4%FCM, kg	4806.74	495.90	10.32	3780	5828
Second lactation					
Milk yield, kg	5539.41	527.16	9.52	4416	7430
Content of milk fat, %	3.94	0.07	1.78	3.72	4.10
Yield of milk fat, kg	218.17	21.91	10.04	177.08	294.97
Yield of 4%FCM, kg	5488.29	537.68	9.80	4423	7397
Third lactation					
Milk yield, kg	5840.71	604.50	10.35	4454	8480
Content of milk fat, %	3.94	0.08	1.96	3.67	4.20
Yield of milk fat, kg	229.88	23.76	10.34	176.82	336.66
Yield of 4%FCM, kg	5784.47	595.72	10.30	4434	8442

Imported animals, in all three lactations realized higher yield of milk (449.08 kg in the first, 568.52 kg in the second and 488.73 in the third lactation), milk fat (19.87 kg, 22.66 kg and 18.52 kg, respectively) and 4% fat corrected milk (477.64 kg, 567.26 kg and 473.36 kg, respectively). When it comes to milk fat from domestic cows, they had lower milk fat content in the first lactation by 0.05%, but higher in the second and third by 0.01%. Differences (Tab. 3) in yield of milk and milk fat, 4% fat corrected milk in favour of imported cattle were highly significant ($p<0.01$), as well as differences in the content of fat in the first lactation, while the differences in the content of fat in the second and third lactation were statistically insignificant ($p> 0.05$).

Lower values of milk yield for imported animals from Germany are reported by *Perišić (1998). Romčević et al. (1990)* found a highly significant difference in milk production, milk fat and milk fat in cows progeny of the same bulls in German population in relation to the production performance of the population in Serbia.

Table 3. Differences in studied milk performance traits between domestic and imported cattle

Indicator	I lact.		II lact.		III lact.	
	difference	t_{exp}	difference	t_{exp}	difference	t_{exp}
Milk yield, kg	-449.08	-7.795**	-568.52	-9.472**	-488.73	-7.524**
Content of milk fat, %	-0.05	-4.502**	0.01	-0.409 ^{ns}	0.01	1.174 ^{ns}
Yield of milk fat, kg	-19.87	-8.519**	-22.66	-9.326**	-18.52	-7.136**
Yield of 4%FCM, kg	-477.64	-8.274**	-567.26	-9.431**	-473.36	-7.336**

* - p<0.05; ** - p<0.01; ^{ns} - p>0.05

Average values and variability of fertility traits in domestic and imported cattle are shown in Tables 4 and 5.

Table 4. Average values and variability of studied fertility traits in domestic cattle (n=245)

Indicator	$\bar{x}$	SD	CV	Min.	Max.
First lactation					
Age at calving, days	765.03	78.68	10.28	652	1129
Duration of service period, days	98.18	50.29	51.22	27	320
Second lactation					
Age at calving, days	1154.73	100.36	8.69	979	1518
Duration of service period, days	93.48	49.49	52.94	30	341
Third lactation					
Age at calving, days	1535.34	113.71	7.41	1283	1930
Duration of service period, days	88.07	33.29	37.79	39	202

Table 5. Average values and variability of studied fertility traits in imported cattle (n=58)

Indicator	$\bar{x}$	SD	CV	Min.	Max.
First lactation					
Age at calving, days	844.16	102.16	12.10	684	1311
Duration of service period, days	111.47	59.53	53.40	40	277
Second lactation					
Age at calving, days	1244.24	118.50	9.52	1023	1701
Duration of service period, days	103.67	59.37	57.26	38	317
Third lactation					
Age at calving, days	1623.97	153.44	9.45	1194	2062
Duration of service period, days	104.02	36.02	34.63	41	244

Domestic cows first calved earlier by 79.13 days, the second time by 89.51 and third time by 88.62 days, and all differences were statistically highly relevant ($p<0.01$). In Table 6, statistically significant differences ($p<0.01$) in the duration of service period can be seen and that the cows from domestic breeding/rearing had a shorter service period by 10.19 (II lactation) and 15.94 days (III lactation). *Croatian Livestock Selection Centre (2003)* in its annual report cites certain reproduction indicators for Simmental cattle population. The average age of registered cows in first lactation was 28 months, in second lactation 39 and the third 53 months. Average duration of service period was 120 days.

By analysing production and reproductive performance of Simmental bull dams in our country, *Pantelić et al. (2005)* have found the average age at first conception of 517.61 days, as well as the service period of 108.98 days.

Table 6. Differences in studied fertility traits between domestic and imported cows

Indicator	I lact.		II lact.		III lact.	
	difference	t_{exp}	difference	t_{exp}	difference	t_{exp}
Age at calving, days	-79.13	-6.479**	-89.51	-5.892**	-88.62	-4.965**
Duration of service period, days	-13.28	-1.744*	-10.19	-1.355^{ns}	-15.94	-3.228**

* - P<0.05; ** - P<0.01; ^{ns} - P>0.05

The results indicate that even though there were statistically significant differences in age at calving and duration of service period between domestic and imported cows in first lactation, these differences did not significantly increase in the second and third lactation. This can mean that the imported heifers were fertilized later, or that they needed some time to adapt and prepare for insemination for the second calving.

Conclusion

Based on the results concerning the effect of import of breeding cattle of Simmental breed on improvement of production traits of domestic cattle population on the territory of Šumadija district, the following can be concluded:

Imported animals, in all three lactations realized higher yield of milk (449.08 kg in the first, 568.52 kg in the second and 488.73 in the third lactation), milk fat (19.87 kg, 22.66 kg and 18.52 kg, respectively) and 4% fat corrected milk (477.64 kg, 567.26 kg and 473.36 kg, respectively). When it comes to milk fat

from domestic cows, they had lower milk fat content in the first lactation by 0.05%, but higher in the second and third by 0.01%.

Differences in yield of milk and milk fat, 4% fat corrected milk in favour of imported cattle were highly significant ($p<0.01$), as well as differences in the content of fat in the first lactation, while the differences in the content of fat in the second and third lactation were statistically insignificant ($p> 0.05$).

All studied cows on average calved the first time at the age of 780.17 days, the second time on 1171.86, and third 1552.31 days, the average service time decreased with the subsequent lactations from 100.73 days to 95.43 days and 91.13 days.

Domestic cows first calved earlier by 79.13 days, the second time by 89.51 and third time by 88.62 days, and all differences were statistically highly relevant ($p<0.01$). Statistically significant differences ($p<0.01$) were established in the duration of service period and it can be concluded that the cows from domestic breeding/rearing had a shorter service period by 10.19 (II lactation) and 15.94 days (III lactation).

From the results obtained in the study it can be concluded that the imports of high-quality heifers of Simmental breed from Germany significantly influenced the improvement of milk and fertility traits in cow population in the Šumadija district.

Efekat uvoza priplodnih krava simentalske rase na poboljšanje proizvodnih osobina domaće populacije

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Rezime

Na osnovu rezultatata ispitivanja efekta uvoza prilpodnih grla simentalske rase na poboljšanje proizvodnih osobina domaće populacije krava na teritoriji Šumadijskog okruga, mogu se izvesti sledeći zaključci:

Grla iz uvoza su u sve tri laktacije ostvarile veću proizvodnju mleka (449,08 kg u I, 568,52 kg u II i 488,73 u III), mlečne masti (19,87 kg; 22,66 kg i 18,52 kg) i 4% mast korigovanog mleka (477,64 kg; 567,26 kg i 473,36 kg). Krave iz domaćeg odgoja imale su niži sadržaj mlečne masti u prvoj laktaciji za 0,05%, ali i viši u drugoj i trećoj za po 0,01%.

Razlike u ostvarenim prinosima mleka, mlečne masti i 4% mast korigovanog mleka u korist uvoznih krava bile su visoko signifikantne ($p<0,01$), kao i razlika u sadržaju mlečne masti u prvoj laktaciji, dok su razlike u sadržaju mlečne masti u drugoj i trećoj laktaciji bile statistički neznačajne ($p>0,05$).

Sve ispitane krave su se u proseku prvi put telile u uzrastu od 780,17 dana, drugi put 1171,86 dana, i treći put 1552,31 dana, prosečan servis period je opadao sa porastom laktacije po redu od 100,73 dana, preko 95,43 dana do 91,13 dana.

Krave iz domaćeg odgoja su se prvi put telile ranije za 79,13 dana, drugi put za 89,51 dan i treći put za 88,62 dana, i sve razlike su bile statistički visoko značajne. Takođe su ustanovljene statistički značajne razlike ($p<0,01$) i u trajanju servis perioda gde su krave iz domaćeg odgoja imale kraće servis periode od 10,19 (II laktacija) do 15,94 dana (III laktacija).

Na osnovu iznetog u zaključku može se konstatovati da je uvoz kvalitetnih priplodnih junica simentalske rase iz Nemačke značajno uticao na poboljšanje osobina mlečnosti i plodnosti populacije krava u Šumadijskom okrugu.

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SOME APPLICATIONS OF ARTIFICIAL NEURAL NETWORKS USED FOR BEEF CATTLE PRODUCTION

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Invited paper

Abstract: An artificial neural network is a system based on the operation of biological neural networks, in other words, is an emulation of biological neural system. It has the capacity to learn, remember, and forget. This is advantageous because the system based on ANNs can update its own efficiency for prediction of the desired output. It is a system based on the operation of biological neural networks and emulation of biological neural system. It is also an adaptive, most often nonlinear system that learns to perform a function from data. Adaptive means that the parameters are changed during operation, called the training phase. After the training phase the ANN parameters are fixed and the system is deployed to solve the problem. The studies were dealing with the application of various methods, such as ultrasound and X-ray methods, digital image analysis, optical probes and magnetic resonance imaging. However, due to high costs, all these methods are not recommended for breeding and production practice. Recent research activities have shown that they are used for mastitis prediction, milk fat and protein prediction, estimation of somatic cell count and fat and protein content in milk, evaluation of a physiological status of cows (estrus, calving and health status) and analysis of in vitro embryo development. ANN models were also developed for predicting and determination of an objective measurement of slaughter value in beef cattle using pre-slaughter information. Therefore, in this reviewed study, some applications of ANN for carcass evaluation are discussed.

Key words: Modelling, Forecasting, Artificial Neural Networks, Animal Production, Carcass

Introduction

Artificial neural networks (ANNs) have been developed that have the capacity to analyze and sort large inputs of data. Because the function of the neural network was patterned after the human brain. The possibilities of accurate predicting cattle slaughter value based on live animal evaluation have been investigated since many years in all over the world. The studies were dealing with the application of various methods, from among the most important are: ultrasound and X-ray methods, digital image analysis, optical probes and magnetic resonance imaging. However, due to high costs, all these methods, besides the ultrasound one, are not recommended for breeding and production practice (*Brethour, 1994*).

Artificial neural networks (ANN), which contains the information about classification and interpretation are used to solve very different problems. ANN modeling technique has been used widely today in many areas. A simple way to mimic the operation of the human brain, ANN has an important place in artificial intelligence studies. "Universal approximation of functions learning method as defined ANN methodology of data, to make generalizations, to operate an unlimited number of variables and it is similar in many important features. These features provide significant advantages in development of prediction models (*Hu et al., 2003*).

Artificial neural networks have been developed that have the capacity to analyze and sort large inputs of data. Because the function of the neural network was patterned after the human brain, it has the capacity to learn, remember, and forget. This is advantageous because the system can constantly update its own efficiency for prediction of the desired output.

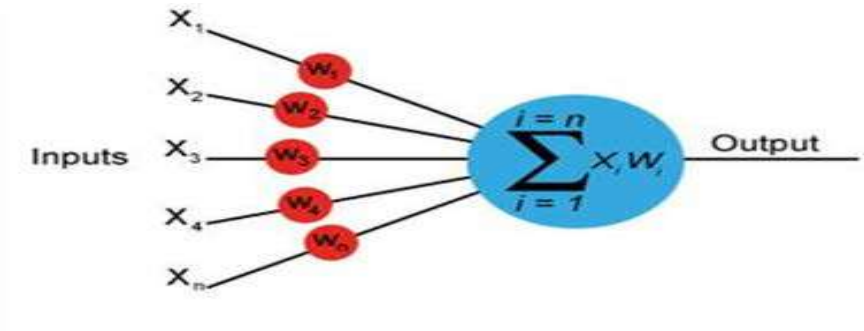

Figure 1. Artificial neuron

Recent research activities in artificial neural net-works (ANNs) have shown that ANNs have powerful pattern classification and pattern recognition

capabilities. Inspired by biological systems, particularly by research into the human brain, ANNs are able to learn from and generalize from experience. Currently, ANNs are being used for a wide variety of tasks in many different fields of business, industry and science (*Bozkurt et al., 2007; Bozkurt et al., 2009).*

In recent years Artificial Neural Networks (ANN) method, together with the non-traditional programming methods is used quite successfully for the solution of difficult or impossible problems in estimating and analyzing the variable. ANN can be applied in various fields such as education and scientific research on livestock feed and transferred to practical, research is still continuing to open up new dimensions (*Arbib, 2003; Hu et al., 2003*).

Many research results, have shown that the marketable value of pre-slaughtered animals can be predicted by using ANN effectively. (*Brethour, 1994; Li et al., 1999; Hill et al., 2000; Adamczyk et al., 2005*).

Some researchers demonstrated that ANN were used effectively in animal husbandry. Some ANN models in estimate the mastitis cases (*Nielen, 1995; Yang et al., 1999; Yang et al., 2000*), in milk composition, estimation of fat and protein yield as well (*Lacroix et al., 1995; et al Wade et al., 1995; Salehi et al., 1998*), the evaluation of the physiological condition of cows (oestrus, calving and health status) (*Vassileva et al., 2001*) and in the analysis of in vitro embryo development (*Wilkinson et al., 1996*).

Materials and Methods

Artificial neural networks, originally developed to mimic basic biological neural systems– the human brain particularly, are composed of a number of interconnected simple processing elements called neurons or nodes. Each node receives an input signal which is the total ‘‘information’’ from other nodes or external stimuli, processes it locally through an activation or transfer function and produces a transformed output signal to other nodes or external outputs. Although each individual neuron implements its function rather slowly and imperfectly, collectively a network can perform a surprising number of tasks quite efficiently (*Torrecilla, 2005*).

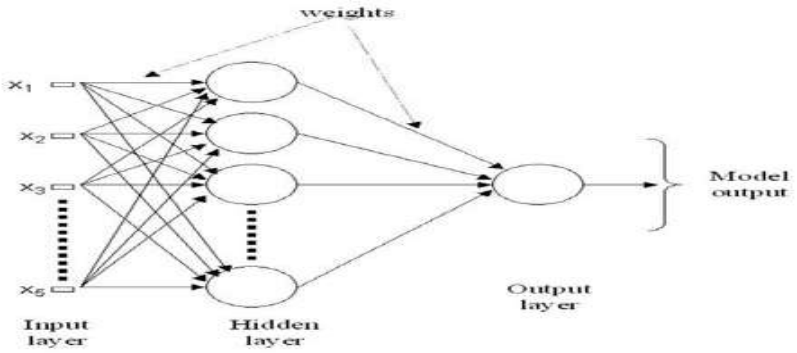

Figure 2. Architecture for 3-layer neural network showing different neurons in each layer

Generally the whole data are turned into sub-groups in Matlab software program and 10% of the data is left as an external validation set 80% / 20% of the remaining 90% data is allocated to the training and test data set of internal network. The data that is allocated for training neural network models and improve internal test data set was used to set the parameters automatically during training the neural network.

The stages of development of an ANN are as follows;
1. General description of the artificial neural network model
2. Validation (validation), training and selection of the test data set
3. The transformation and analysis of data
4. The selection of variables
5. Network formation and training
6. Testing the validity of the model with external data sets

ANN softwares (Predict v2, ImagePro v6, Matlab v7) are used to simulate research, develop and apply artificial neural networks, A typical ANN consists of layers. The first layer is known as inputs layer (sex, age, breed, LW at slaughter etc.) and the last one is called output layer (Model output for predicted values for carcass quality) (*Bozkurt et al., 2009)*. All the other layers are called hidden layers. The ANN training then is necessary for model prediction ability. For this purpose, all input and output values are normalized to have a zero mean and a unified standard deviation (using MATLAB's prestd function), and finally outputs are

transferred back to the same units as the original outputs (using MATLAB's poststd function). After confirming the input and target of the network, then network optimization is conducted in order to increase prediction accuracy *(Fausett, 1994).*

Implications

Many authors found a high effectiveness of ANNs application in cattle breeding. They have been used for mastitis prediction, milk, fat and protein yield prediction, estimation of somatic cell count and fat and protein content in milk, evaluation of a physiological status of cows (oestrus, calving and health status) and analysis of in vitro embryo development. Neural network models were also developed for predicting and determination of an objective measurement of slaughter value in beef cattle using pre-slaughter information.

Conclusion

In the evaluation of the results obtained from a research based on determination of carcass quality the data are expose to image processing and neural network modeling. The relationship between body and carcass measurement parameters can be examined by obtained correlation coefficients; prediction equations by the regression analysis. Both live animals as well as carcass measurements predictions can be obtained through digital image analysis and artificial neural network. The live weight and hot carcass weight are used to estimate performances of the animals. The estimated live weight and carcass weight can be determined by regression analysis. The results then are compared with those obtained by ANN applications.

In conclusion ANN applications will certainly open up new dimensions in animal production practices.

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Neke aplikacije veštačkih neuronskih mreža u govedarstvu

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Rezime

Veštačke neuronske mreže predstavljaju sistem zasnovan na radu bioloških neuronskih mreža, drugim rečima, predstavljaju emulaciju biološkog nervnog sistema. Imaju sposobnost da uče, zapamte i zaborave. To je prednost, jer sistem zasnovan na ANN (veštačke neuronske mreže) može da ažurira svoju efikasnost za predviđanje željenog izlaza. To je sistem zasnovan na radu bioloških neuronskih mreža i emulaciji biološkog nervnog sistema. Takođe je prilagodljiv, najčešće nelinearni sistem koji uči da obavlja funkciju na osnovu podataka. Adaptivan – prilagodljiv znači da se parametri menjaju tokom rada, koja se zove faza obuke. Nakon faze obuke ANN parametri se fiksiraju i sistem je upotrebljen odn. stavljen u rad da reši problem. Studije su se bavile primenom različitih metoda, kao što je ultrazvuk i rendgen-metoda, digitalna obrada slike, optičke sonde i magnetne rezonance. Međutim, zbog visokih troškova, sve ove metode se ne preporučuju za uzgoj i proizvodnu praksu. Skorašnje istraživačke aktivnosti su pokazale da se oni koriste za predviđanje/procenu mastitisa, mlečne masti i proteina, procenu broja somatskih ćelija i sadržaja masti i proteina u mleku, procenu fiziološkog stanja krava (estrusa, telenja i zdravstveno stanje) i analize in vitro razvoju embriona. ANN modeli su takođe razvijeni za predviđanje i određivanje objektivnog merenja klanične vrednosti kod tovnih goveda koristeći podatke pre klanja. Dakle, u ovom preglednom radu, razmatraju se neke aplikacije ANN za ocenu trupova.

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EMERGING DISEASES AND ZOONOSES: GLOBAL RISK FOR ANIMAL AND HUMAN HEALTH

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Invited paper

Abstract: The emergence of new infectious diseases in the 20th is linked to increasing contact between humans, animals, and intensification and integration of food production. Animals are the dominant factor implicated in the occurrence of infection and development of the diseases. Globalisation and climate changes have a huge impact in the world on the occurrence and development of infectious diseases in humans and animals. Furthermore, changes in demography, people's behavior, technology progress, international transportation and trade, but also changes and adaptation of microorganisms, are all important factors for the occurrence of infectious diseases. These diseases are a consequence of emerging new pathogens or changed ones that have already existed before. Intensive communication and trade with animals and agricultural products provided conditions for rapid transmission of the disease throughout the world. Infectious diseases arise as a consequence of mutual contact between humans and domestic and wild animals, as well as of environmental changes. To the purpose of preventing disease transmission and potential negative outcomes, comprehensive research and adequate actions are of vital importance. Multidisciplinary approach and mutual acting of veterinarians, physicians, biologists, agronomists and ecologists, is the prerequisite for successful addressing this issue. Veterinary medicine should play crucial role in the future of the One Health concept. The professionals and expert in this field must follow global trends affecting animal health and diseases and improve their knowledge and expertise to effectively address future challenges.

Key words: emerging diseases, zoonoses, animal health, one health

Introduction

Globalization and climatic changes have a wide range of impacts on the occurrence and development of infectious diseases, both human and animal ones. Moreover, the changes in human interactions and demography, technology advancement, international transport and trade as well as changes and adaptability of microorganisms are important factors for the occurrence of infectious diseases resulting from the emergence of new pathogens, alterations of known pathogens as well as changes in the distribution and prevalence of known pathogens among new host population or territories.

Infectious diseases profoundly influence the health status of a population and economy in every country, affecting both humans and animals; however, the poorest (developing) countries commonly suffer the most severe consequences. The rate of disease emergence and spread is difficult to predict, having in mind a wide range of important factors implicated in the mechanism of the disease. In that respect, records and analyses of previous epidemics as well as monitoring and updating of control and disease surveillance programmes using advanced technologies (satellite measurements) and new scientific accomplishments are highly relevant, as well as preparing of some potential intervention strategies in case of disease emergence. Human and veterinary service institutions should take effective steps and adequate measures for the prevention and control of infectious diseases and the development of new methods for early, rapid and accurate diagnosing (nanotechnologies, biosensors, etc.)

According to the World Health Organization (WHO), *"Health is a state of complete physical, mental and social well-being and not merely an absence of disease or infirmity"*. Good health is the fundamental right of all people on Earth.

"One Health" has been defined as "the collaborative effort of multiple disciplines — working locally, nationally, and globally — to attain optimal health for people, animals and the environment". One Health is an interdisciplinary model integrating diverse disciplines such as medicine, veterinary medicine, public health, environment protection, wildlife protection, ecology, and food safety aimed at forming unified solutions applicable for the improvement of the health of humans, animals and the environment. Such an approach proved the best policy for health protection at global level.

Emerging infectious diseases

The emergence of new infectious diseases in the 20th is linked to increasing contact between humans and animals, intensification and integration of food

production, and the expansion of international travel (*Atlas 2003*), as well as intensive climatic changes. Animals are the dominant factor implicated in the occurrence of infection and development of the diseases, thus, of the 1,415 microbes that are known to infect humans, 61 percent come from animals (*Taylor et al, 2001*). Consequently, and in light of some previous incidents a group of health professionals at Rockefeller University in New York developed the slogan "One World - One Health" with an aim of promoting and recognizing the association of animals and environmental factors and their effects on human health.

The avian influenza (HPAI H5N1) epidemic that began in Hong Kong in 1997, forced the global community to recognize that animal health and human health are closely linked. The HPAI H5N1 virus resurfaced in isolated outbreaks, and in late 2003, severe epidemic of highly pathogenic avian influenza was registered in South Korea. Delays in international reporting and poor public reaction in the region resulted in extensive virus spread across the region of Southeast Asia. As a response to the global threat posed by this pathogen, the Food and Agriculture Organization (FAO), World Health Organization (WHO), and World Organization for Animal Health (OIE) developed a strategy and tripartite agreement on mutual activities aimed at preventing infections related with the animal-human-ecosystem interface (*FAO 2013*). One Health is therefore an ideal model of joining health practitioners, veterinarians, ecologist and other profiles. Improvement of epidemiology centres and services as well as application of modern molecular methods will contribute to the development of effective measures and strategies for successful implementation of health protection programs worldwide (*Atlas 2003*).

Emerging diseases and zoonoses

Discovery and wide application of antibiotics and vaccination programs have long been considered to have fully eradicated the infectious diseases, thus putting the emphasis on health protection programs aimed at cardiovascular and non-infectious diseases. On the contrary, during the past few years we have been witnessing the emergence of new infectious diseases in both humans and animals, as well as the diseases that were considered eradicated or under control. The most important diseases, which represent a global health problem, include Human Immunodeficiency Virus (HIV), Severe Acute Respiratory Syndrome (SARS), West Nile virus, highly pathogenic avian virus H5N1, and recently identified influenza virus H1N1. The underlying cause of this phenomenon is complex and multifold (*Brown 2004, Morse 2004*) including close contacts between wild and domestic animals and humans, intensive livestock husbandry, inadequate biosafety measures in livestock production practice, emerging resistance of bacteria towards

antibiotics, intensive people mobility and interactions, international trade, transport, changes of the eco-systems, etc.

Communication/mobility

Current air traffic enables reaching almost every place on Earth within 36 hours, which is much less than the incubation period for many of viral and bacterial diseases, suggesting the possibility of rapid global distribution of the infection even before the manifestation of a single clinical symptom. Consequently, the disease outbreaks can occur in the regions in which they have never been recorded before. Thus, West Nile virus was not known in North America before 1999, but it is widely known at almost all continents. This strongly suggests that health professionals and veterinarians should be acquainted with the epidemiological and clinical aspects of the diseases, which are still uncommon in their countries, and be able to recognize them. Such diseases encompass some highly contagious diseases such as foot and mouth disease, rinderpest (cattle plague), African swine fever, atypical avian pest, highly pathogenic avian influenza virus, etc. (*Jones 2008*).

Modern communication and OIE health information system enabled fast flow of information of outbreak and spreading of infections, enabling identification of disease reservoirs and implementation of rapid and effective control measures by the veterinary service. Moreover, relevant information on the spread of the infection and measures (aimed at prevention and elimination of consequences) taken by the responsible authorities will be available to the public. In that respect, veterinary service must prepare effective and adequate plans and control strategies including adequately trained staff and communication resources to minimize potential hazards for human and animal health. Lack of proper coordination within the entire system for health protection can result in severe impact on the safety of foods of animal origin and the consumers, as well as substantial financial losses to the producers.

International trade

Dramatic increase in goods turnover on international market during the past few decades resulted from the efforts to remove the barriers and enable the access to world markets to all participants in the international trade. The global trade of animals and products of animal origin has enormously expanded as the result of reduction or elimination of import taxes as well as the advancements in the technology of storage and preservation of food. It is also linked with an increase in global consumption associated with the improved living standards, especially in economically developed countries.

Since the 1960, global meat production has tripled, milk production doubled and production of eggs is almost four times higher (*Speedy, 2003*). Intensive turnover and movement of animals and foods is associated with increased risk of the transmission of infectious diseases, above all food borne zoonozes. Considering the increased risk, WTO (World Trade Organization) has taken an active role in the development of regulations and policies aimed at protecting animal health and ensuring food safety as the key elements of international trade. OIE provides scientific support on animal turnover regulation to WTO, whereas FAO and WHO (via *Codex Alimentarius*) provide the guidelines to the regulations pertaining to food products, including foods of animal origin.

Wars and terrorism

War is a restraining factor that influences animal health in many aspects. It may lead to failure of specific programs for disease control as well as total collapse of veterinary service and infrastructure. War conflicts cause serious problems related to food production, movement of refugees together with their animals to other regions. Bioterrorism added new and worrisome dimension in the process of disease control. The majority of potential agents used so far are of zoonotic nature, thus, both humans and animals would be endangered in case of biological warfare (*Davis 2004*).

Changes of the climate and other environmental factors

The relationship between the animal husbandry and environment and health is a bi-directional interaction. On one hand, animals may have negative effects on the environment, while on the other hand, animal health and productivity are greatly affected by the same ecological burden (*Sherman 2010*). Ruminants are an important source of gaseous emission, contributing to global warming. Livestock production has become an important factor in environmental degradation through deforestation, particularly in Latin America. In regions with intensive livestock production, excess animal waste (manure and slurry) may cause pollution of water streams. Overgrazing results in soil degradation, especially in semi-arid regions. At the same time, human and animal diseases can result from environmental impact or climatic changes. Global warming is associated with spread of Bluetongue disease in Europe *(Purse et al. 2005*), Hantavirus infection in North America, malaria epidemics in Africa and South America, Dengue fever in Asia and cholera in South Asia (*Anyamba et al 2006*).

The role of a veterinarian in the One Health concept

There are several phrases and terms associated with One Health such as "united health", integrated health, one medicine, one world-one medicine; however, disregarding the definition, this concept should represent a global strategy of multidisciplinary collaboration in all aspects of human and animal health protection, and environment protection (*Gibbs 2013, Vidic et al 2014a*). Such a multidisciplinary approach should be accomplished at local, national and global levels to provide adequate protection of human and animal health. Global trends and numerous technological advancements from the past few decades have contributed to increased development and worldwide spread of zoonotic diseases. In spite of substantial social advantages associated with globalization, it also posed a range of challenging issues in view of domestic animals, human health and environment protection.

Veterinary medicine should play crucial role in the future of the One Health concept. The professionals and expert in this field must follow global trends affecting animal health and diseases and improve their knowledge and expertise to effectively address future challenges (FAO 2013).

Veterinarians are the only health professionals whose formal training is based in comparative medicine, with in-depth studies of the health and diseases of multiple species (*Chaddock 2012, Pal 2014*) Veterinarians are also trained in public health as it pertains to livestock production and environmental assessments associated with animal health problems and diagnosis (water, feed, and air quality, etc...). Veterinarians are active in public health by working closely with physicians and other health professionals on disease investigation, surveillance and effective response to potential disease outbreaks (*Hoblet 2002, Pal 2014, Vidic et al 2014b*). Veterinarians in the zoo and wildlife sectors are acquainted with wild animal diseases, thus able to be a part of interdisciplinary teams composed of wildlife biologists, ecologists and other specialists (FAO 2013, Gibbs 2013).

Consequently, the veterinary medical profession needs to be actively involved in the One Health approach with an aim of human, animal and environmental well-being. Potential barrier for veterinarians and students of veterinary medicine might be the identification of career pathways and employment opportunities for working on One Health issues in a global context (*Chaddock 2012*). In addition to technical competence, other important attributes are strong leadership abilities, teamwork, foreign language proficiency, improved communication skills, cross-cultural experience, adaptability and flexibility and even advanced training in the other medical specialty areas such as epidemiology, toxicology or virology.

Conclusion

Veterinary medicine should play crucial role in the future of the One Health concept. The professionals and expert in this field must follow global trends affecting animal health and diseases and improve their knowledge and expertise to effectively address future challenges. As a consequence of modern technologies, some local changes or incidents may result in effects of global importance. This is particularly significant in case of disease outbreaks, since current globalization era is associated with increased number and incidence of infectious diseases, especially zoonoses. Intensive communication and trade with animals and agricultural products provided conditions for rapid transmission of the disease throughout the world. Infectious diseases arise as a consequence of mutual contact between humans and domestic and wild animals, as well as of environmental changes. To the purpose of preventing disease transmission and potential negative outcomes, comprehensive research and adequate actions are of vital importance. Multidisciplinary approach and mutual acting of veterinarians, physicians, biologists, agronomists and ecologists, is the prerequisite for successful addressing this issue.

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Preteće bolesti i zonoze: globalni rizici za zdravje životinja i ljudi

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Rezime

Pojava novih zaraznih bolesti u 20 veku posledica je povećane komunikacije između ljudi, životinja i intenziviranja proizvodnje hrane. Dominantan uticaj na pojavu i nastanak bolesti imaju životinje. Globalizacija i klimatske promene imaju ogroman uticaj u svetu na nastanak i razvoj zaraznih bolesti ljudi i životinja. Osim toga promene u demografiji i ponašanju ljudi, napredak tehnologije, međunarodni transport i trgovina, kao i promene i adaptacija

mikroorganizama su značajni faktori za pojavi zaraznih bolesti, kao posledica pojave novih patoge na, izmenjenih već postojećih patogena i poznatih patogen koji su dokazani na novim području ili novoj populaciji domaćina. Povećan nivo komunikacije i trgovine životinjama i robama stvorilo je uslove da se bolest može veoma brzo preneti na sve krajeva sveta. Infektivne bolesti nastaju kao posledica kontakta ljudi, domaćih i divljih životinja i promena u životnoj sredini. Da bi se ove posledice predupredile potrebno je temeljno izučavanja i delovanje u tom sistemu, da bi se bolesti mogle prevenitrati. To zahteva multidisciplinarni pristup, ili zajedničkog delovanja i povezivanja veterinara, lekara, biologa, agronoma, ekologa i drugih.

Veterinarska medicina u budućnosti treba da ima veliki značaj u konceptu Jedno zdravlje. Veterinarsli stručnjaci moraju pratiti globalne trendove koji utiču na zdravje i bolesti i usklade neophodna znanja i stručnost da bi bili efikasni u budućim izazovima.

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WELFARE AND BEHAVIOUR IN RELATION TO DISEASE OF DAIRY COWS

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Invited paper

Abstract: In this paper different aspects of welfare and behaviour in relation to disease of dairy cows were discussed. Diseases that affect dairy cows in literature were commonly classified as organ-related, reproductive, metabolic, infectious and parasitic. The special significance, both for the extensive and intensive dairy production, has diseases of the hooves, as well as mastitis and ketosis. Understanding the aetiology of the disease or syndrome is important for detecting potential animal welfare problems. Sick cows are manifested sickness behaviour that controlled by cytokines of the immune system and appear to be part of the immune response to the illnesses. It is well known fact that sick cow changes normal behaviour patterns in many aspects and its well-being are more or less compromised depending from diseases severity. Cow respond to illness with a consistent and predictable pattern of behavioural changes in reactivity, exploration, ingestion, reproduction, social interactions, body hygiene maintenance, movement, territoriality, rest and sleep depending from nature of disease. Automated detection of cow health and behaviour problems provides a complement, and perhaps eventually an alternative to the current use of farmers' records or records of veterinary treatment to assess the animal welfare.

Key words: behaviour, dairy cows, disease, welfare

Introduction

Determination of the health status in long term period was considered as the useful indicator of dairy cow welfare, although scientists in this very important

field often were paid little attention to the connection between the problems of the animal health, behaviour and welfare. For this approach there are numerous reasons, and as most important are difficulties in accomplishment data on the incidence of health problems and in the similar sense behavioural disturbances in different types of dairy cow production. It is well known fact that information on cow health are available and significantly easier to establish in extensive than in intensive dairy rearing systems due to smaller number of the animals in the first system. Further problems that scientists were faced were different incidence and prevalence of disease and their relevance as indicators of the welfare of dairy cows (*EFSA, 2009a; Rushen et al., 2008; von Keyserlingk et al., 2009; Hristov and Stanković, 2014*). Despite noticeable progress in dairy cow welfare today, there are many unsolved problems in the use of health indicators in assessing of that very important issue. Since there are many illnesses in dairy cow that reduce the welfare as main question were appointed on-farm assessment of the relative impact of different forms of the disease on cow welfare and thereby difficulties associated with establishing reliable and valid information on the occurrence of the disease (*EFSA, 2009a; von Keyserlingk et al., 2009; Stanković et al., 2014a*).

Dairy cows suffer from many diseases, both in intensive and extensive production systems (*EFSA, 2009a; Hristov et al., 2012a; Stanković et al., 2014a*). These animals can suffer from many different endemic diseases, such as infectious and non-infectious, and occasionally diseases with epidemic character. Diseases that affect dairy cows in literature were commonly classified as organ-related, reproductive, metabolic, infectious and parasitic. From organ-related diseases the most significant in dairy production are indigestion (meteorism, rumen acidosis and rumen alkalosis) and from reproductive diseases very frequently encountered ovarian cysts, metritis and retained placenta (*EFSA, 2009a; Stanković et al., 2014a; Stanković et al., 2014b*). Among metabolic diseases, that common affect dairy cow, in intensive production systems, the most important are allotriophagy, ketosis and puerperal paresis. Major importances among parasitic diseases for dairy cows worldwide have babesiosis, fasciolosis, gastrointestinal strongiloides, hypodermosis and scabies. Finally from infectious diseases in cow worldwide are important bovine spongiform encephalopathy, foot-and-mouth disease, tuberculosis and brucellosis. It should take into account that infectious and parasitic diseases can exist in enzootic or epizootic forms. The special significance, both for the extensive and intensive dairy production, has diseases of the hoofs, as well as mastitis and ketosis (*EFSA, 2009a; OIE, 2008*).

It is well known fact that sick cow changes normal behaviour patterns in many aspects and its well-being are more or less compromised depending from diseases severity (*EFSA, 2009a*). It is also well known fact that certain normal behaviours of dairy cows may contribute to occurrence of metabolic, parasitic and

infectious diseases, and thus jeopardize their well-being (*Broom and Fraser, 2007; Rushen et al., 2008*). Poor welfare of cows, on the other hand, contributes to a number of diseases and evokes changes of certain behaviour patterns in order to adapt to the harmful effects of diseases (*Broom and Fraser, 2007; Rushen et al., 2008*). In this relationship also it is obvious that the illness of the animals can be a major cause of poor welfare, and that the occurrence of diseases can be used to assess the welfare of dairy cows (*Broom and Corke, 2002; Broom, 2006*). It is considered that rearing conditions have great impact on health and welfare of dairy cows (*Fregonesi and Leaver, 2001; Regula et al., 2004; Hristov et al., 2008; EFSA, 2009a; Stanković et al., 2014a*), reproductive and related disorders (*Stanković et al., 2014b*) and milk traits (*Hristov et al., 2014b*)

Nowadays records on the incidence and prevalence of various diseases in dairy cow are becoming more readily available because of on-farm record keeping systems. Today it is considered that stockman need to be able to correctly recognize specific cow health issues and behavioural disturbances early in order to enhance its welfare, as well as the cow health status of their herd (*EFSA, 2009a; Hristov and Stanković, 2014*).

The aim of this review paper were detailed consideration of the connection between welfare, behaviour and cow diseases as well as outline of the behaviour and welfare in relation to the most important diseases in intensive dairy production.

Definition of dairy cow welfare, behaviour and health

When scrutinizing relationship between welfare, behaviour and health of the cow then, first of all, it should have in mind the definition of all of these states. According to *WHO* (*1948*) health is a state of complete physical, mental and social well-being and not merely the absence of disease or infirmity.

According to *OIE* (*2008*) animal welfare means how an animal is coping with the conditions in which it lives. In addition, an animal is in a good state of welfare if, as indicated by scientific evidence, it is healthy, comfortable, well-nourished, safe, able to express innate behaviour, and if it is not suffering from unpleasant states such as pain, fear, and distress.

Welfare of dairy cow indicates a state in which the biological functions of its body are within the normal range, or this is a state in which the animal are completely mentally and physically healthy and in harmony with its living environment (*Broom and Fraser, 2007; Rushen et al., 2008; Hristov and Stanković, 2014*). Protecting of cow welfare is considered in terms of defining indicators of well-being and ratings individual level of welfare quality. In this regard, as the most important indicators, in literature, were taken into account many physical, physiological and behavioural indicators of cow health (*Fregonesi*

and Leaver, 2001; Broom and Fraser, 2007; Winckler, 2008; Rushen et al., 2008; Hristov et al., 2012b; Hristov et al., 2014a).

When it is necessary to consider the welfare of dairy cows in terms of definition and assessment of the indicators, scientist paid special attention to the animal-based indicators and especially emotions of the animals reflecting mental status and also the quality of its life (*Broom and Fraser, 2007; Winckler, 2008; Rushen et al., 2008; Hristov and Stanković, 2014*). At the same time, scientist considered that indicators of the presence of positive well-being are pleasant emotions as comfort, satisfaction and feeling safe and negative well-being unpleasant emotions such as fear, pain, suffering, feeling threatened, etc. In addition, many scientists estimated biological functions of the animal body, such as normal growth and development of the animal, reproductive traits, the expression of physiological behaviours, and absence of disease and behaviours disturbances as very important indicators of cow welfare (*Rushen et al., 2008; Hristov et al., 2014a*).

Behaviour can be defined as a set of observable activities or reactions of the cow to stimuli that originate from the body and from the environment in order to adapt to the conditions of life and survive in a certain environment. Many basic dairy cow behaviour activities are of vital importance for the survival in a particular environment, such as reactivity, exploration, ingestion, reproduction, social interactions, body hygiene maintenance, movement, territoriality, rest and sleep which were described by many authors (e.g. *Vučinić, 2006; Broom and Fraser, 2007; Rushen et al., 2008; Hristov and Stanković, 2014*).

In cases where the dairy cow cannot cope with the conditions in which they live in many occasions, different behaviour disturbances include self-mutilation, pathological reactivity, redirected behaviour and stereotypes. Besides this, in intensive dairy rearing conditions often occur states of suffering that is contrary to cow welfare. Suffering is unpleasant emotion that is usually caused in cows by pain, fear or distress, discomfort, insecurity, deprivation of basic needs, prolonged hunger and thirst, frustration and conflicts. In fact, cows suffering represents failure or inability of individuals to adapt and to overcome the factors that cause a feeling of mental exhaustion (*Vučinić, 2006; Broom and Fraser, 2007; Rushen et al., 2008*).

Presuming that individuals welfare is consequence of animals attempts to cope with environment and pathology is one of the effects of environment, then it is clear that health is a part of welfare (Broom, 2006). One should have in mind that the pathology is the detrimental derangement of molecules, cells and functions that occurs in living organisms in response to injurious agents or deprivations (*Broom and Corke, 2002; Broom, 2006*).

Sickness behaviour

Biological basis of the sick animals behaviour was described in detail by *Hart* (*1988*). The roles of cytokines in sickness behaviour were presented in review scientific papers by *Kelley et al*. (*2003*) and *Dantzer* (*2004*). In these papers, it was stated that sickness behaviour is mediated by proinflammatory cytokines that are temporarily expressed in the brain during infection. Somewhat earlier in the review paper by *Aubert* (*1999*), it was summarized that behavioural changes induced by cytokines are not merely the consequence of a degraded state but reflect motivational reorganization.

Sickness behaviour and its relevance to animal welfare assessment at the group level were considered by *Millman* (*2007*). Behaviours shown by ill animals are part of a coordinated strategy to fight disease. The behavioural changes attributable to illness are controlled by cytokines of the immune system and so appear to be part of the immune response to illness. A better knowledge of sickness behaviour in cow will help to improve the ability to detect illness earlier, especially when the animals are housed in large groups.

Certain researches, especially that demonstrate the role of fever in facilitating an animal's ability to combat viral and bacterial infections, have led to the development of a viewpoint that the behaviour of a sick individual is not a maladaptive and undesirable effect of illness but rather a highly organized behavioural strategy (*Hart, 1988; Aubert, 1999*). The behavioural repertoire of animals' changes dramatically follows infection. Essentially, animals respond to illness with a consistent and predictable pattern of behavioural changes, which typically include reduced feeding, increased rest and sleep, increased thermoregulatory behaviours and reduced social behaviour (*Hart, 1988*). In other words, expressed sick individuals have little motivation to eat, they are listless, complain of fatigue and malaise, loose interest in social activities and have significant changes in sleep patterns. They display an inability to experience pleasure, have exaggerated responses to pain and fail to concentrate. These conditions are a consequence of proinflammatory cytokines acting in the brain cause sickness behaviours (*Kelley et al., 2003*). The behavioural changes that occur when animals are ill are not simply a by-product of the debilitating effects of illness, but organized behavioural changes that help animals recuperate from the illness (*Hart, 1988; Aubert, 1999*). Clinical diagnose of illness often involves some objective assessments of behaviour changes. These behavioural changes occur simultaneously with physiological and metabolic changes, most notably the fever response, and some of the behavioural changes serve to maintain the fever (*Johnson, 2002*).

The relationship of the health, behaviour, and welfare of cows

Health problems in dairy cows are best correlated with the welfare of these animals in relationship to aspects of the animal suffering in the past, present or future (*von Keyserlingk et al., 2011*). Understanding the aetiology of the disease or syndrome is important for detecting potential dairy cow welfare problems. Morbidity rates, including for infectious and metabolic diseases such as mastitis and metritis, lameness, metabolic diseases, parasitic diseases and postpartum complications and injury rates, above recognised thresholds, may be direct or indirect indicators of the animal welfare status of the whole herd (*OIE, 2008*).

As mentioned before, links between cow welfare, behaviour and severity of diseases can be observed through the duration and the probability that the disease will cause death. Another approach that could be used in that sense is to establish common symptoms in different diseases. Symptoms such as decrease food intake, decrease of general activity, lying and similar behavioural changes that occur in a number of diseases could be used, so that the relative severity of the disease can be assessed by comparing the relative extent of these changes. Also, diseases can be ranked based on the combined loss of milk production and reduce food intake (*Broom and Fraser, 2007; Rushen et al., 2008; von Keyserlingk et al., 2011*).

Because of the increasing incidence of various diseases, and the large economic cost for dairy production, there is a huge interest in early detection of illness. Automated detection of animal-health problems provides a complement, and perhaps eventually an alternative to the current use of farmers' records or records of veterinary treatment to assess animal welfare (*González et al., 2008*). Useful information about connectivity options between behavioural changes and health disorders can be found in the papers that have scrutinised disturbances of rest behaviour of dairy cows (*Chaplin 2000*), hooves lesions and behaviour in pregnant and early lactation heifers at housing (*Chaplin et al., 2000*), possible link between behaviour and the occurrence of lameness in dairy cows (*Galindo et al., 2000*), especially relationships between social behaviour and the occurrence of lameness (*Galindo and Broom, 2000*) and the effects of lameness on social and individual behaviour of dairy cows (*Galindo and Broom, 2002*), feeding behaviour and health disorders (*González et al., 2008*), prepartum feeding behaviour as early indicator of subclinical ketosis (*Goldhawk et al., 2009*), prepartum behaviour and dry matter intake as risk for metritis (*Huzzey et al., 2007*), and lying behavior as an indicator of lameness in dairy cows *Ito et al.* (*2010*). Several studies have shown that feeding behaviour can change before clinical signs of disease are evident (*Huzzey et al., 2007; González et al., 2008; Goldhawk et al., 2009*). These

researches show that monitoring feeding behaviour may give producers a better chance of detecting transition cow illness at an early stage. Certainly, automated monitoring of feeding behaviour can be helpful in detecting illness (*Sorensen et al., 2002; Svensson and Jensen, 2007*). There are many diseases that afflict cows during calving and transition, including dystocia, metritis, metabolic disorders (as ketosis, fatty liver etc), displaced abomasum and milk fever. Despite decades of research, the incidence of these diseases in intensive dairy production remains unacceptably high. Changes in cow behaviour during transition can predict many disease and lameness risk post partum (*von Keyserlingk et al., 2011*).

Dystocia

Dystocia is common disorder with clear implications for dairy caw welfare. Effects of a difficult calving on the subsequent health and welfare of the dairy cows and calves were well documented by *Barrier* (*2012*) in her PhD thesis and by *Barrier et al*. (*2012*) in research paper that considered effect of a difficult calving on the vigour of the calf, the onset of maternal behaviour, and some behavioural indicators of pain in the dam. The effect of dystocia on the dam welfare ranges from discomfort because of laceration of the vulva to paralysis of the obturator nerve and downer cow syndrome (*Egan et al., 2001*). In the literature, there are consensus that calving difficulty or at least severe difficulty at calving has adverse effects on the health, behaviour and production of the dairy cow (*Barrier, 2012*). Dairy cows that have dystocia are more likely to develop health complications after calving, reducing productivity and welfare (*Proudfoot et al., 2009; Barrier, 2012; Barrier et al. 2012*).

Metritis

Metritis can have long-term effects on dairy cows, substantially reducing both milk production and feed intake (*Bareille et al., 2003*), indicating a relatively severe impact on cow welfare.

Huzzey et al. (*2007*) found that cows with diagnosed metritis spent less time feeding during the precalving period than did their healthy counterparts; every 10-min decline in prepartum feeding time was associated with a 2-fold increase in the chance that a cow would be diagnosed as suffering from metritis. These results indicate that changes in feeding behaviour can be a sensitive indicator of this disease.

Downer cow syndrome

Very common disorder in dairy is downer cow syndrome, which namely denotes many conditions that cause cows to lie down on their chest or side and remain unable to rise. About calving time, causes of this syndrome can be grass

tetany, milk fever, pregnancy toxaemia, acute acidosis, calving paralysis and toxic mastitis. Detailed information relating to all of these complex pathologies in cows can be found in the publication by *Radostits et al*. (*2007*).

Ketosis

Changes in prepartum feeding behaviour as an early indicator of subclinical ketosis were described in the paper by *Goldhawk et al*. (*2009*). Ketosis is a metabolic condition that occurs when a cow is in negative-energy balance immediately after calving. Peak prevalence of subclinical ketosis occurs in the first two weeks of lactation. To support the cows' energy demands, the body must mobilize fat reserves resulting in the production of ketone bodies by the liver. This condition increases the cows' risk of displaced abomasum, fatty liver, retained placenta, reduces milk production, and decreases reproductive performance. Ketosis in dairy cow can cause a range of signs. Wasting form of ketosis manifest rapid loss of condition 2 -8 weeks after calving, drop in milk yield, poor appetite and breath smells strange (like acetone) due to the presence of ketones. In nervous form in cows occur incoordination, strange behaviour e.g. sucking items in the environment, wandering aimlessly and the cows may become aggressive (*Duffield, 2000; Radostits et al., 2007*).

Mastitis

One of the most important infectious diseases in dairy cows is mastitis because of the high prevalence, poor welfare and economic losses caused. Risk assessment of the impact of housing, nutrition and feeding, management and genetic selection on udder problems in dairy cows were described in detail by *EFSA* (*2009b*). Evidence suggests that cows with this disease show sickness behaviours, but little is known about the progression of behavioural changes before and after the disease becomes clinical. Changes in behaviour of dairy cows with clinical mastitis were illustrated in the paper by *Sepúlveda-Varas et al.* (*2014*). Cows with naturally occurring clinical mastitis in early lactation showed typical sickness behaviours, including decreased feed intake and a reduction in competitive behaviour at the feeder. Dry matter intake gradually decreased in the days before diagnosis, and recovered one day after treatment. Competitive interactions tended to decrease before diagnosis, and increased after treatment, albeit at a slower rate than the feeding behaviours.

Lameness

Lameness is widely regarded as a major welfare problem for dairy cows. The influence of the environment on dairy cow behaviour, hooves health and herd lameness dynamics were illustrated in detail by *Cook and Nordlund* (*2009*). Impact

of lameness on behaviour and productivity of lactating Holstein cows were described by *Juarez et al.* (*2003*). This health problem results in decreased mobility, reduced dry matter intake, decreased production, impaired reproduction, debilitated cows and early culling. Also, *Proudfoot et al*., (*2010*) have determined that behaviour during transition differs for cows diagnosed with claw horn lesions in mid lactation. Review of behaviour of lame cows was documented by *Olechnowicz and Jaskowski* (*2011a*). Lameness, activity time-budgets and estrus expression in dairy cattle are described by *Walker* (*2008*). Comparisons of hoof lesions and behaviour in pregnant and early lactation heifers at housing were given *by Chaplin* (*2000*). Relation between clinical lameness and reproductive performance in dairy cows were described in detail in the paper by *Olechnowicz and Jaskowski* (*2011b*). In this paper, authors stated that lameness of cows is an important factor influencing the longevity and performance of the herd, including milk yield losses and also that it has a negative impact on reproductive performance and constitutes an increasing risk for early culling.

Effect of lameness on the behaviour of dairy cows under intensive production systems were reviewed by *Varlyakov et al*. (*2012*). Based on literature data, these authors determine that cows with movement problems and found lameness losing position in the food trail, changing his place of rest, losing the position to enter the milking room or visits of milking robot, which changes the whole order of entry for milking. The effects of lameness on social and individual behaviour of dairy cows were studied by *Galindo and Broom* (*2002*). They found that the lame cows spent more time lying out of the cubicles, had longer total lying times, and spent less time feeding. The behavioural differences described in this paper show that lame cows do not cope as successfully with their environment as do non-lame cows. Also, authors were ascertained that no differences were found in the times licking other cows; however, the frequency of times being licked was higher in the lame cows. Although lame cows were less likely to start an aggressive interaction, there were no differences in times receiving aggression.

Lameness indicates a painful state and discomfort and is regarded as one of the most serious welfare problems in cattle. It is listed under behaviour related parameters and can be linked to disturbances in the cows' laying down behaviour, but is also clearly linked to animal diseases in terms of claw diseases, and as such, the condition leads to severely changed behaviour in the cow. Behavioural changes associated with lameness were described in detail by *EFSA* (*2009a*). Lameness in dairy cows presents a major welfare problem whether assessed in terms of their physical state or mental state There are many welfare problems attributable to lameness. These include infertility, loss of production, premature culling, pain and other behavioural indices of discomfort or distress. Recognition of lameness as a major welfare problem both in European legislation and in quality assurance

programmes operating within the dairy industry gives emphasis to the assumption that all foot lameness is an expression of pain that may vary from slight to severe (*EFSA, 2009a*).

Undernutrition and overnutrition

Body condition scoring in cow can be performed using a variety of scales and systems. Body condition score and its association with dairy cow productivity, health, and welfare were given by *Roche et al*. (*2009*). Associations among dairy cow body condition and welfare-associated behavioral traits were described by *Matthews* (*2012*). In cow both undernutrition and overnutrition can be regarded as a potential welfare problem, since cows which are overconditioned at drying off are more likely to develop cystic ovarian disease and lameness. Severe body condition loss from the dry to near calving period increased the occurrence of retained placenta. In addition, too thin animals may be regarded as welfare relevant per se, since they have obviously not been able to meet their physiological demands and may suffer from prolonged hunger.

Conclusion

According literature data about welfare and behaviour in relation to disease of dairy cows and experience of authors, it can be concluded:

- Behaviour, welfare and disease occurrence in dairy cows are closely related and conditioned states;
- Sick cows are manifesting sickness behaviour that controlled by cytokines of the immune system and appear to be part of the immune response to illness;
- Dairy cows respond to illness with a consistent and predictable pattern of behavioural changes in reactivity, exploration, ingestion, reproduction, social interactions, body hygiene maintenance, movement, territoriality, rest and sleep related to nature of disease;
- Morbidity rates, including for infectious and metabolic diseases such as mastitis and metritis, lameness, metabolic diseases, parasitic diseases and post-partum complications and injury rates, above recognised thresholds, may be direct or indirect indicators of the cow welfare status of the whole herd;
- Automated detection of cow health and behaviour problems provides a complement, and perhaps eventually an alternative to the current use of farmers' records or records of veterinary treatment to assess the animal welfare.

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Dobrobit i ponašanje u odnosu na bolesti mlečnih krava

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Rezime

U radu su prikazani različiti aspekti dobrobiti i ponašanja analizirani u odnosu na bolesti mlečnih krava. Oboljenja koja se javljaju kod mlečnih goveda se u literaturi obično dele na organska, reproduktivna metabolička, infektivna i parazitska. Poseban značaj, kako u ekstenzivnoj tako i u intezivnoj proizvodnji, imaju bolesti papaka, mastitis i ketoza. Poznavanje etiologije bolesti ili sindroma je važno za razumevanje mogućih problema dobrobiti mlečnih krava. Bolesne krave ispoljavaju ponašanje karakteristično za takvo stanje organizma, a koje je pod kontrolom citokina imunog sistema i smatra se da takvo ponašanje predstavlja deo imunog odgovora na bolest. Poznato je da ponašanje obolele krave odstupa u različitim aspektima od normalnog obrasca ponašanja, a njena dobrobit je ugrožena u manjoj ili većoj meri, u zavisnosti od toga koliko je oboljenje ozbiljno. Krava reaguje na bolest odstupanjem od uobičajenog i predvidivog obrasca ponašanja u pogledu reaktivnosti, istraživanja, ishrane, reprodukcije, socijalne interakcije, održavanja higijene tela, pokreta, teritorijalnosti, odmora i sna, u zavisnosti od prirode bolesti. Automatsko otkrivanje problema zdravlja i ponašanja krava obezbeđuje dodatne podatke, kao i alternativu aktuelnoj upotrebi podataka vođenih na farmi ili podataka o lečenju za procenu dobrobiti životinja.

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HOUSING CONDITIONS AND WELFARE OF DAIRY COWS IN SERBIA

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Invited paper

Abstract: Research conducted on 16 dairy farms in Serbia has shown that poor housing conditions represent one of the major dairy cows' welfare problem in our country. This is also an area of animal welfare in which the greatest difference in relation to the situation in EU countries can be observed. Poor comfort conditions are estimated based on a high share of cows that lie outside their lying area (36.5%) as a consequence of inadequate or insufficiente size of lying areas. Investigated the farms showed a very poor state of hygiene of dairy cows, with a high percentage of cows with the contaminated lower parts of the legs (84.6%), rump (71.3%) and udder (60.0%), which indicates the inadequate hygiene of lying areas and facilities, insufficient amount of bedding but also disorders of rumen digestion. The biggest welfare problems are present on farms with tied system, also the presence of grazing in our conditions, unlike the EU, is also insufficient from the standpoint of ensuring the welfare of dairy cows.

Key words: welfare, dairy cows, housing conditions, comfort, freedom of movement, hygiene

Introduction

The general concept of welfare is based on the concepts of adaptation, stress, animal needs and their rights. The most important question in terms of providing welfare is certainly the question of animal needs. According to *Broom and Johnson (1993)* the need is the request, part of the biological basis of the animal, to provide adequate resources or responses to specific stimuli from the surrounding environment or its body. Animals in the absence of resources to meet their basic needs are becoming more prone to numerous welfare risks. In the report,

EFSA (2009) highlighted four key risks to the welfare of dairy cows: housing, feeding, management and genetic selection. The conditions for housing of dairy cows, according to research by other authors (*Regula et al., 2004; Ostojić - Andrić et al., 2011)* significantly affect the ensuring of their welfare and include a wide range of conditions that need to be provided in order to ensure the animal needs and protect welfare. The authors of the *Welfare Quality Protocol (Welfare Quality®, 2009),* as key factors to ensure satisfying of the animal needs in regard to housing conditions, include freedom of movement and comfort of the animals.

As a form of behavior, movement is an integral part of all other forms of behavior and enables the animal to react appropriately in terms of space, time and stimulus which meets their needs for maintaining the homeostasis, or physical, psychological and genetic integrity (*Vučinić, 2006*). If the animal is highly motivated to satisfy a need, and due to the inability of movement is prevented in doing so, it leads to the development of frustration *(Vučinić, 2006).*

According to many authors (*Krohn and Munksgaard,* 1993; *Ristov et al., 2006; Ostojić - Andrić et al., 2011*), the housing system is strongly reflected on the quality of the welfare of dairy cows, especially in terms of health status and expression of behavior. In Serbia, like in most countries, the tied system prevails that allows individual treatment of each animal but is a strong contrast to the natural habitat of the cattle (*Ostojić - Andrić et al., 2011*). Free system is more and more present in modern rearing because the freedom of movement and separation of functions (feeding, watering, lying and milking) have a positive influence on the overall state of health, fitness of the animal, length of exploitation and production performance. Easier way to ensure the proper microclimate and zoohygienic conditions are also benefits of the free system.

Growing cows in the pasture system is the most natural form of rearing of cattle since it enables the expression of various forms of behavior characteristic of the cattle as a species (*Von Keyserlingk et al., 2009*). *Tucker (2009)* states that cattle grazing have more harmonious daily rhythm compared to farmyard kept cows, and that their daily feeding and lying pattern is better synchronized. The advantages of growing cattle on pasture are reflected primarily in lower incidence of laminitis, teat injuries (*Regula et al., 2004*) and abnormal behaviors (*Krohn, 1994*) as well as enhanced comfort while resting (*Krohn and Munksgaard, 1993*).

Providing comfort to cows is of great importance from the aspect of meeting their needs in terms of rest, behaviour and health. Studies have shown that the hygiene of cows and their behavior on the laying area during their resting can be considered as reliable indicators of comfort.

Hygiene of cows mirrors the environment in which they are grown and influences many aspects of health (*Schukken et al., 1990*). Some studies the behaviour of cows show that they have an aversion to unclean environment,

especially when first dealing with it, and that it is preferably avoided (*Phillips and Morris, 2002).* Soiling of various bodily parts of cows shows the different sources of contamination. For example, dirty lower legs suggest that cows are walking through mud or manure, whereas dirty hips suggest dirty and damp lying areas. Specific, splattered manure patterns on the hips are a sign that a cow kicked with a tail that was dirty with faeces and watery. This combination of behaviour and consistency of feces can be an indicator of disturbed function of the rumen (*Huxley and Whay, 2006).*

Lying is the behavior of high priority (*Munksgaard et al., 2005*), because the cows mainly rest during lying, so every change of this aspect is of great significance for the state of welfare. In studies *Brörkens and al. (2009a)* have found that the duration/time of lying down (in seconds), the percentage of collision with equipment when lying down and the percentage of cows that lie partially or completely outside the lying are can be considered as the most reliable indicators of welfare.

Taking into account the above-mentioned research in this field in the world, and the need for assessing the state of welfare on dairy farms in our country, the main objective of this study was to determine the quality of welfare in relation to housing conditions in order to define the most important welfare risks and propose measures for its improvement.

Materials and Methods

For the assessment of welfare in dairy farms in Serbia the protocol for evaluating the quality of the welfare of cattle (*Wefare Quality® Assessment Protocol for Cattle, 2009*) was used, specifically designed to assess relevant indicators of welfare from the viewpoint of the animals themselves. The protocol includes 29 indicators that are used to determine the 12 criteria: the absence of long-term hunger and thurst, comfort, thermal comfort, freedom of movement, lack of injuries and illness, absence of pain due to mutilation, expressing social and other behaviours, good human - animal relations and a positive emotional state. By aggregation of these criteria the values of 4 basic principles of welfare are determined: good nutrition, good housing, good health and appropriate behavior. Finally, based on the overall score farms are classified into one of four categories of quality of welfare: unacceptable, acceptable, appropriate and excellent. The study was conducted on a total of 16 farms with different housing (tied, free) and different capacity (small, medium, large) in which the cattle of Simmental and Holstein - Friesian breeds were reared. Minimum number of cows in the sample was 30 and the average per farm was 64 animals in two repetitions - during winter and summer season. Data processing and categorization of welfare quality of the

investigated dairy farms was conducted using software specially developed under the Protocol, and the respective statistical parameters were analyzed with the program *StatSoft.Inc (2004), Statistica for Windows version 7*.

Results and Discussion

Overall welfare assessment on dairy farms in Serbia

Welfare quality assessment on dairy farms was conducted by collecting data relating to the ensuring of good nutrition, good housing, good health and appropriate behaviour. The research results show that half of the surveyed farms was classified as acceptable (score 2) and the other half into the category of welfare of adequate/appropriate quality (grade 3) on the basis of which it can be argued that the observed farms, on average provided conditions that are of fundamental animals' needs in terms of nutrition, health, comfort, housing and expression of behaviour. Research conducted according the same methodology in farms in the EU (*Welfare Quality Network, 2012*) showed a great similarity with the results obtained in the present study. In 2011, the share of farms with an acceptable quality of welfare in the EU was 47%, with 51% of the appropriate quality and 2% of farms with unacceptable quality of welfare, while in our country none of the evaluated farms were classified in the latter category.

Table 1 shows the average rating of the quality of the welfare of the analyzed farms. The principle of good nutrition is rated satisfactory with the highest score in relation to other principles which indicates that the welfare of dairy cows in Serbia is not threatened by long-term starvation and thirst. Under this principle, the highest variability was established, so in certain farms (score $\leq$ 20) shortcomings were evident which, given the importance of this principle, need to be timely removed. Sums of other principles on average were in the range from 20 to 55 points, which corresponded to category of acceptable welfare, i.e. indicated that housing conditions, health and behaviour requirements were ensured and meet the minimum needs of animals in terms of their welfare. However, the survey results indicate that there is room for improvement, especially when it comes to housing conditions and the provision of appropriate behaviour.

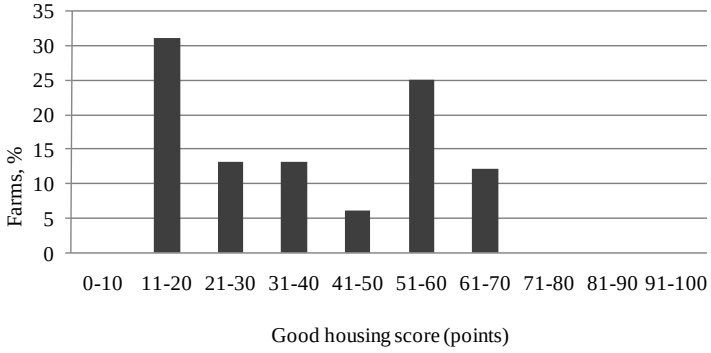
Table 1. Principle scores and overall assessment of welfare quality on dairy farms in Serbia

WF*	Welfare principles, score	$\bar{x}$	*SD*	S^2	*Min*	*Max*
En	Good feeding	75,97	25,31	640,39	12,20	100,00
Ac	Good housing	36,59	19,37	375,18	7,30	65,40
Ac	Good health	41,17	8,11	65,78	23,90	56,60
Ac	Appropriate behaviour	31,93	13,77	189,50	15,40	81,10
Overall welfare assessment (1-4), average score		2,47	0,51	0,26	2,00	3,00

*Welfare category (WF): Ex-excellent (>80 points); En-enhanced (55-80 points); Ac-acceptable (22-55 points) and Nc-not classified (< 20 points)

Welfare quality in relation to housing conditions

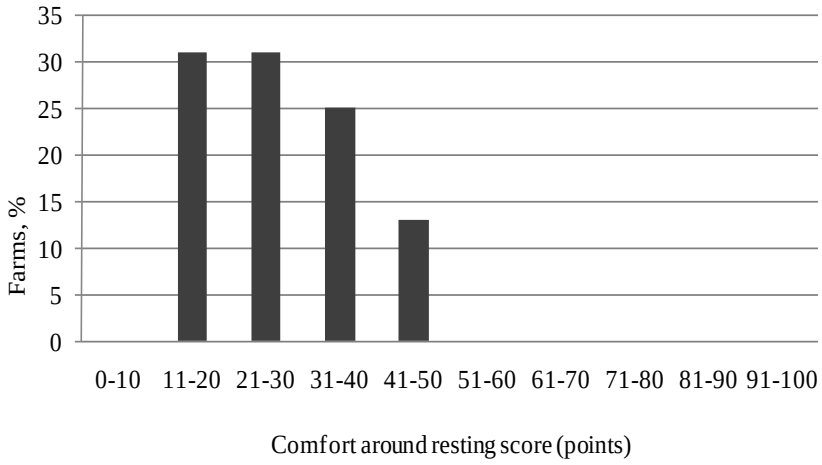
Distribution of the examined farms in Serbia according to the value of the principles of good housing (Graph 1) shows that the highest number of dairy farms (31.25%) was rated as very poor, in the range from 11 to 20 points, and the remaining 68.75% of the farms was rated in the range from 21 to 70 points. The average value of the principle was 36.59 points compared to the score of this principle in EU countries (*Welfare Quality Network, 2012*) where the highest number of farms (50%) is estimated in the range from 51 to 70 points with an average principle value of 56.5 points. It is obvious that dairy cows in our country are provided with significantly poorer growing/housing conditions compared to those that exist in European farms. This observation is further confirmed by the fact that in our study, no farm is estimated to be in the range from 81 to 100 points whereas in the EU 2% of farms are within these values. Also, only 2% of the farms in the EU are valued in the range from 11 to 20 points, in contrast to Serbia, where the highest number of farms are estimated within this range.



Graph. 1. Distribution of farms according to principle of good housing

Farms in Serbia on average were mostly (62.5%) scored in the range from 11 to 30 points for the criterion of comfort in housing (Graph 2), with an average value of 25.77 points as opposed to farms in the EU (*Welfare Quality Network, 2012*) where the average value was 45.1 points on 44% of farms scored in the range from 31 to 40 points and 4% estimated in the range from 1 to 10 points. This indicates that the comfort conditions in the housing of cows in Serbia on average is worse than the conditions that are provided for cows in EU countries.

In relation to the value of criterion freedom of movement, for the highest number of farms in the survey – 43.75% (Graph 3) were very highly rated (90 to 100 points), which means that the animals are provided with adequate capacity to move in stables, ranges or on pastures. However one third of the surveyed farms was estimated in the range from 11 to 20 points, which indicates a significant limitations in terms of freedom of movement.

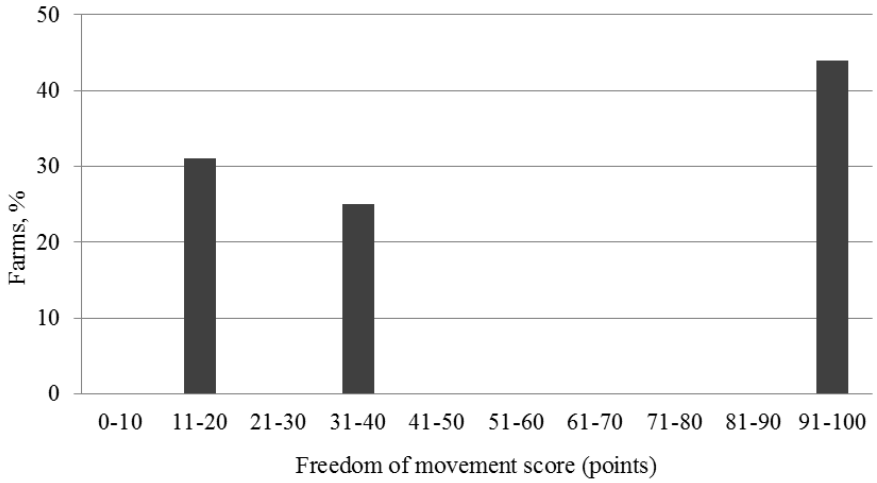


Graph. 2. Distribution of farms according to comfort around resting

Results of research of freedom of movement on dairy farms in the EU indicate that this parameter is significantly improved in the past five years. In 2008, the share of farms with a score of freedom of movement in the range from 11 to 20 points was 18%, while in 2012 all studied farms had the highest value of the criteria of 90 to 100 points (*Welfare Quality Network, 2012*).

Housing/rearing conditions are factors of great importance for the cow welfare. The value of the principle of appropriate growing conditions on farms in Serbia is defined by the values of criteria comfort and enabled freedom of movement (Table 2).

Survey results (Table 2) show that the cows in tested farms on average are given greater freedom of movement than is the case with the provision of comfort. It was determined that the average duration of lying down of 6.25 seconds is outside the range of acceptable values according to recommendations *Forkman and Keeling (2009)* and indicates the limited comfort of cows due to inadequate lying areas, problems with diseases of legs and pathological changes on the skin, hair and joints (*Algers et al., 2009*).



Graph. 3. Distribution of farms according to Freedom of movement

The collision with the equipment when resting was below the critical value of over 20% as recommended by *Forkman and Keeling (2009),* while the phenomenon of lying outside the lying area was recorded at an average 36.54% of cows which seriously undermines the comfort of cows. In addition, cows lying partially (in tied) or fully (in the free system) out of the lying area has a negative impact on their hygiene, behaviour and health (*Algers et al., 2009*). The most common cause of this phenomenon in the tied system are inadequate and short lying areas (*Bartussek, 1985*) but also attempts of cows to cool in the slurry during the summer months in case of free range system *(Curtis, 1982).*

Hygiene of cows on observed farms was very poorly scored (Table 2). An average proportion of cows with dirty lower parts of the legs, the hips and udders far exceeds the critical ranges in terms of the welfare of 20 - 50%, 10 - 19% and 10 - 19% respectively (*Forkman and Keeling, 2009*). The share of cows with dirty feet (Table 2) of 60% and 95% was very close to the share identified in the research of *Vučemile et al. (2012).*

Cook (2002) has identified inadequate - short lying areas and insufficient amount of bedding on them as the most common causes of high soiling of the lower parts of the legs. In fact, a high proportion of cows with dirty feet is not uncommon even on the farms of high welfare quality category.

Table 2. Assessment of housing conditions in relation to welfare assurance

No of farms, N	N=16				
Principles, criterions and indicators of welfare quality	$\bar{x}$	*SD*	S^2	*Min*	*Max*
II Good housing, score	36,59	19,37	375,18	7,30	65,40
1. Comfort around resting, score	25,77	12,50	156,32	2,70	45,10
Time needed to lie down, in sec.	6,25	0,72	0,52	4,50	7,58
Colliding with housing equipment during lying down, %	9,93	11,58	134,13	0,00	37,00
Lying partly or completely outside the lying area, %	36,54	33,83	1144,38	0,00	100,00
Dirty legs,%	84,64	20,39	415,77	14,81	100,00
Dirty udders,%	60,07	23,99	575,54	9,26	100,00
Dirty flank/ upper legs,%	71,34	20,42	417,05	3,70	100,00
2. Freedom of movement, score	56,94	39,25	1540,58	15,00	100,00
No. of days cows are kept tethered, per year	174,84	170,29	28997,56	0,00	365,00
No. of days with access to outdoor loafing area, per year	107,66	120,49	14517,72	0,00	365,00
No. of hours with access to outdoor loafing area, daily	9,00	10,11	102,19	0,00	24,00
No. of days with access to pasture, per year	16,88	52,76	2783,47	0,00	210,00
No. of hours with access to pasture, daily	2,25	6,42	41,23	0,00	24,00

In the study by *Webster (2005*), the ratio mentioned ranged on farms from 65% to 96%. However, in the same survey, filthy rumps were observed in 0 - 7% of cows from the best farms and in 26 -78% of cows from lowest scored farms while in our study, this ratio was 71%.

Soiling of the udders on farms in Serbia amounted to an average of 60%, which corresponds to the range of 24 - 70% on farm of the worst welfare quality category in the research of *Webster (2005*). In a study of *Vučemila et al. (2012)* the

soiling of rump was even more pronounced (75%). Extremely poor hygiene of cows usually is associated with poor facility hygiene and ninadequate and irregular manure removal but may be associated with disfunction of the rumen (*Huxley and Whay, 2006).* Soiling of rump can be a reliable indicator of the said conditions. It can be concluded that inadequate hygiene of cows on farms in Serbia threatens their welfare by increasing the risk of diseases of legs and udders (*Schukken et al., 1990)* while at the same time it can cause changes in the behaviour of cows (*Phillips and Morris, 2002*).

The movement is an essential element for expression of natural behaviours and satisfaction of innate need or instinct of animals. In our conditions the cows are, on average, held tied 175 days per year while access to ranges is enabled 108 days and pastures only 17 days (Table 2). Converted into hours, cows spend outdoors a total of 968 hours per year on free ranges discharges or 38 hours in the pasture. It can be concluded that the time cows are in the outdoors (in ranges and in the pasture) is very short and insufficient, taking into account the survey by *Krohn et al. (1992)* where cows, with the possibility of free choice, each year spent 4046 hours in the open air.

In our research, freedom of movement was acceptable, but with large variation (min. 15; max. 100) between the analyzed farms. On six of the sixteen examined farms, tied system of keeping was applied throughout the year which represents a major risk to the welfare of animals and reflects negatively on their comfort while resting (*Krohn and Munksgaard, 1993*) and the state of health of cattle in terms of increased incidence of laminitis and mastitis (Regula et al., 2004), as well as abnormal behaviours *(Krohn, 1994).*

Conclusion

Average score of the welfare quality on the tested farms corresponds to the descriptive assessment acceptable to appropriate welfare which leads to the conclusion that rearing/housing conditions satisfy more of the basic needs of animals in terms of nutrition, health, comfort, housing and expression of the behaviour, and there is room for improvement of welfare. Similar results were found on dairy farms in the EU.

Conditions of keeping/housing of dairy cows in Serbia were deemed acceptable, but still significantly worse than the conditions that exist on European farms. On the tested farms, cows on average were given greater freedom of movement than is the case with the provision of comfort. Indicators of comfort in keeping point to significant problems with hygiene in the facilities, insufficient bedding and too short lying areas. Freedom of movement in the average was assessed as adequate. The biggest problems are certainly present on farms with tied

system, where on more than half of the farms cows were not given possibility of movement during the year. Representation of grazing in our conditions, unlike the EU, is also insufficient from the standpoint of ensuring the welfare of dairy cows.

Results of testing the quality of welfare in relation to housing conditions, generally indicate that the quality of the welfare on the studied farms is acceptable and appropriate, and that there are opportunities for improvement. With regard to the identified major risk factors and current trends in the dairy cattle production, recommendations for improvements in welfare are related to the provision of adequate space and comfort in keeping/housing of dairy cows, the provision of adequate size, build quality and hygiene of lying areas as well as providing greater freedom of movement of cows through the application of free housing system and grazing.

Uslovi držanja i dobrobit mlečnih krava u Srbiji

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Rezime

Istraživanje sprovedeno na 16 mlečnih farmi u Srbiji pokazalo je da loši uslovi držanja predstavljaju jedan od najznačajnijih problema dobrobiti mlečnih krava u našoj zemlji. Ovo je ujedno oblast dobrobiti u kojoj postoje najveća odstupanja u odnosu na stanje u zemljama EU. Nezadovoljavajući uslovi komfora procenjeni su na osnovu visokog učešća krava koje leže van ležišta (36,5%) kao posledice neodgovarajuće veličine odnosno prekratkih ležišta. Na posmatranim farmama utvrđeno je veoma loše stanje higijene krava, sa visokim učešćem krava zaprljanih donjih delova nogu (84,6%), sapi (71,3%) i vimena (60,0%) što ukazuje na neodgovarajuću higijenu ležišta i objekata, nedovoljnu količinu prostirke ali i poremećaje buražnog varenja. Najveći problemi dobrobiti prisutni su na farmama sa vezanim sistemom držanja dok je i zastupljenost ispaše u našim uslovima, za razliku od zemalja EU, takođe nedovoljna sa stanovišta osiguranja dobrobiti mlečnih krava.

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RESULTS OF FOLLICULOGENESIS INDUCTION AND PRODUCTION OF EMBRIOS FROM SAANEN GOATS

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Invited paper

Abstract: In embryo transplantation, there are some subsequent manipulations, of which the induction of polyovulation in donors is one of the most important elements. At that, the main problem consists in variability of ovary response to treatment. The suggested scheme can be used to induce the polyovulation in donor goats for embryo transplantation with highly synchronized ovulation that does not depend on an initial hormonal pattern of the donor. Use of an ear implant "Crestar" to prolong the luteal phase of the sexual cycle and synchronization hunting of goats is an effective biotechnological technique. It testifies that progestogen from the ear implant actively secreted into the bloodstream, its concentration in the peripheral blood was sufficient for inhibition of FSH output by posterior pituitary gland. Hormonal stimulation scheme folliculogenesis, based on the use of drugs "Ovagen", "Folligon" and "Chorulon" the proposed scheme provides optimal ovarian response with an average level of multiple ovulation of 11,4 yellow bodies per animal at average recovery embryos 81,4% and 8,7 suitable for further manipulation of embryos per donors. As a result of 11 operations 96 cells (cells obtained percentage was 81.4%) are got. It should be noted a large variability of these cells development stages. Thus of the estimated 96 cells 10 cells (9.6%) were unfertilized, 64 cells (66.6%) were in stage of 2 pronucleuses, 22 cells (22.9%) were in stage of 2 and 4 blastomeres.

Key words: goats, sexual cycle, luteal phase, implant, superovulation, yellow body, egg, pronucleus, embryo

Introduction

Embryo transplantation technology is included into the long-term breeding programs for selection, improvement and preservation of existing breeds of agricultural animals in many countries of the world (*Айбазов, 1998*). Profound

researches of the reproductive function in animals, its possible regulation, microsurgical and genetically engineered manipulation with embryos showed that the method of transplantation can be the basis for accelerated reproduction of highly productive animals and whole populations. Practical application of this method in animal husbandry provides intensive reproduction of animals with high genetic value (*Мамонтова et al., 2014*).

In recent decades, the method of transplantation has received further perfection in connection with the dynamic development of transgenic technologies and getting animals with the set properties (*Maxwell et al., 1990*).

Goats are the most perspective object for researches in the field of transgenic technologies for following reasons: they have a rather short (5 months) reproductive cycle, high multiple pregnancies and require minimal care and keeping. Moreover, they maximally correspond to the main requirement at carrying out of genetic engineering works: to production of the necessary proteins, enzymes with a natural biological liquid, that is milk (*Sugie et al., 1980; Айбазов et al., 2013*).

The key moment in the technology of transplantation is working out of an effective way to induce the development of polyovulation. High variability in response rates of polyovulation at its induction with exogenous drugs in goats is a negative factor. Even at identical schemes of hormonal treatment of the animals, that are in identical conditions of keeping and feeding, various ovarian responses are often observed (*Мамонтова et al., 2014; Armstrong et al., 1983; Freitas et al., 2003; Lee et al., 2000; Maurer et al., 1986; Maxwell et al., 1990*).

Own researches in this area have been directed on studying of the effectiveness of various gonadotropic preparations applications to induce the polyovulation in donor goats. The second equally objective was to obtain fertilized eggs at the stage of two pronucleuses and finding-out of factors affecting the effectiveness of it.

Experimental work on biotechnological complex of FGBNU VNIIOK consisted of the following stages: hormonal treatment of donor animals for the purpose of the sexual cycle synchronizing and the induction of polyovulation, surgical washout of zygotes and their evaluation.

Materials and methods

In the experiment conducted in October, 2013, 12 donor goats have been selected. To exclude pregnancy within 25 days, the animals were tested for presence of estrus. After estrus fixation, the animals were placed in a separate cage and left intact.

Hormonal treatment was reduced to following. For synchronization of the sexual cycle, to all donor goats was introduced ear implant "Crestar" (Intervet, Holland) in the lateral ear surface (between the skin and cartilage). Each implant contains 1.5 mg of norgestamet. The ear implant was introduced for a period of 11-12 days. Simultaneously with the introduction of ear implant, all donor animals were injected intramuscularly once with a solution of the drug "Crestar", containing also 1.5 mg of norgestamet and 1.9 mg of estradiol.

To induce the ovaries polyovulation, differing schemes of hormonal treatment have been applied, for what animals were randomly divided into 2 groups.

Goats of the first group (n=6) 60 hours prior to extraction of the ear implant we started to administer intramuscular the preparation of "Ovagen" (an extract of follicle stimulating hormone (FSH) from the pituitary gland of sheep; OVAGEN, "Immunochemical Product Ltd", New Zealand). The application scheme consisted of eightfold hypodermic injection with an interval of 12 hours at equal doses of 0.7 mg. The total dose of FSH for one animal treatment has made 5.6 mg. Simultaneously with the first injection of FSH was injected also the preparation of "Folligon" ("INTERVET", Holland) at a dose of 500 units.

For animals treatment in the second group (n=6), was also used a follicle stimulating hormone (FSH pituitary, USA), but in other form and according to other scheme. Under this scheme FSH-p was administered hypodermically starting 60 hours prior to implant extraction. FSH administration regiment consisted of 3-day treatment with two-fold daily injection, with an interval of 12 hours, under the following scheme: 1^{st} day -5 mg; 2^{nd} day – 4 mg; 3^{rd} day – 3 mg per one injection. The total dose of follicle stimulating hormone has made 24 mg.

Immediately after the extraction of ear implant at all goats was conducted a sampling in heat for what have been used vasectomized teaser bucks with aprons tied up. To determine the exact start time of the heat, sampling was conducted by each 2 hours. To synchronize the ovulations, dams selected in the state of estrus were injected intravenously with 150 units of the drug "Chorulon" (human chorion gonadotropin, hCG) ("INTERVET", Holland).

Artificial insemination was conducted triple since 4^{th} hour from the estrus beginning, for what 0.1-0.2 ml of high quality sperm was introduced intracervical (mobility not less than 8 points, concentration not below 2.5 milliard/ml).

Results and discussion

Prolongation efficiency in the luteal phase of the sexual cycle (ear implants of "Crestar") in a dose of 1.5 mg of active substance with a single injection of norgestamet solution in a dose of 1.5 mg and estradiol in a dose of 1.9 mg was

high. All 16 goats (100%) showed no signs of heat throughout all the treatment period. It testifies that progestogen from the ear implant actively secreted into the bloodstream, and its concentration in the peripheral blood was sufficient for inhibition of FSH output by posterior pituitary gland.

After implant extraction (i.e., termination of the inhibitor) all treated goats came in heat within 24 hours. Donor goats came in sexual heat on the average in 16 hours (limit of 2...24 hours) after extraction of an ear implant. Thus, from 12 goats 4 (33.3%) have come in heat within 8 hours after release of progestogen, 5 (42%) – during the period of 8 to 16 hours and only 3 (15%) – during the period of 16 to 24 hours.

Thus, it was found that for the prolongation of the luteal phase of sexual cycle and for heat synchronization in Saanen goats using of ear implants “Crestar” is an effective biotechnological method.

As to duration of sexual heat, the average lasting of estrus in donor goats has made 21.5 hours, suggesting that the hormone treatment reduces the heat duration.

Polyovulation intensity was determined by the results of fresh yellow bodies’ visualization by laparoscopy using endoscopic equipment “Karl Storz” (German). In the presence of multiple ovulation in the ovaries have been performed the laparotomy, determined the number of yellow bodies in each ovary with subsequent washing out of cells.

It was found out that from the total number of treated animals (n=12) on the average, irrespective of the treatment scheme, 91.7% of goats have responded with multiple ovulation. In the first group the polyovulation was registered at 6 goats from 6 (100%), in the second group – at 5 goats from 6 (83/3%).

Abstraction of embryos was performed by laparotomy after 60-72 hours of the ear implant extraction. It was found that the average number of the ovulations at one positive donor has made 10.8 at variability from 6 to 24. Thus, the polyovulation level in the first group has averaged 11.9 ovulations on one donor (limit of 8 to 16), in the second group it was 10.6 ovulations on one donor (variability of 6 to 24).

The conducted experiment has revealed the high efficiency of tested schemes of the superovulation induction. At the same time, we consider the use of the drug “Ovagen” preferable, as firstly, it provides a higher level of ovulations, and secondly, at its application the variability of ovarian response decreases.

It should be especially emphasized that in both groups of goats, specified schemes of exogenous hormones application did not lead to negative side effects: it was not matured, but not burst follicles, ovarian cystic degeneration.

Operative access to internal genital organs for the embryos extraction in donor goats was performed by the laparotomy under general anesthesia. A cut with

a length of 5-7 cm was done cranial from the edge of the mammary gland on the white line of the abdomen. Through an incision in the abdominal cavity index and middle fingers were introduced, found the uterus and took it carefully outside together with the ovaries. After inspection of the ovaries and registration of the ovulations number (by counting the number of the yellow bodies) washing away of embryos was carried out.

As a washed liquid we used phosphate saline buffered solution of Dulbecco or HEPES-KSOM.

For washing out of embryos polystyrene catheter was used, which was introduced into the oviduct through its funnel, and it was fixed by fingers. By needle of the system for embryos washing out we did a puncture in the wall of the corresponding uterine horn on the greater curvature, closer to the oviduct, while the fingers of the other hand we pinched the uterus horn cavity behind a puncture preventing the flow of washing liquid into the other part of uterus. By syringe of the system we pumped up the washing liquid (30-50 ml), so that through the top of horn it came into the oviduct. The washing liquid with embryos was collected from side of the oviduct in plastic test tubes. After washing the uterus was set into the abdominal cavity, the wound was sutured in layers.

As a result of 11 operations, 96 cells (cells obtained percentage was 81.4%) are got. It should be noted a large variability of these cells development stages. Thus of the estimated 96 cells, 10 cells (9.6%) were unfertilized, 64 cells (66.6%) were in stage of 2 pronucleuses , 22 cells (22.9%) were in stage of 2 and 4 blastomeres.

Table 1. Results of estrus synchronization and polyovulation induction in goats

Number of order	Investigated indicators	Group 1	Group2
1	Number of goats	6	6
2	Animals synchronous came in heat, head, %	6 100	6 100
3	The duration of estrus, hours	20.5	22.5
4	Animals answered with polyovulation, head, %	6 100	6 83.3
5	Number of the operated goats	6	5
6	Level of polyovulation, u/1 donor	11.9	10.6
7	Got cells, overall including on 1 donor	52 8.66	44 8.80

One of the main problems in the embryo transfer technology is the unpredictability of the hormone treatment results in animals in order to induce multiple ovulation and difficulties in exact definition of time of zygotes washout from the oviducts of donor animals. It is of particular importance for goats. Despite a considerable quantity of works carried out in different countries on a large experimental date (using a variety of schemes of polyovulation induction and drugs chosen for hormonal treatment), till now the procedure for getting goat zygotes suitable for transplantation or performance of gene engineering manipulations (for example, microinjections of recombinant DNA) continues to remain key (*Ryan et al., 1992; Sugie et al., 1980).*

For example, researches have noted that even with good effect of superovulation, it is seldom possible to get regularly more than three zygotes at donor goats, suitable for microinjections and transplantation.

The high-lights to the greatest degree influencing the efficiency of fertilized eggs production in the stage of two pronucleuses, according to these authors, are time of heat coming after implant extraction and time interval, within which it is necessary to inseminate donor goats (Гольдман *et al.., 2012).*

At the scheme of hormonal treatment used by us, heat in donor goats came on the average of 16 hours after extraction of ear implant, however this index had quite substantial (± 8 hours) individual variations. It should be noted that the heat in some donor goats began at once after extraction of an ear implant. For this reason, the coming of heat at donor goat we recommend to check the first time during the ear implant extraction, and then every 2 hours before registration of estrus.

Further, based on the average time of heat duration we determined time for covering of females. It is known that duration of heat in various breeds of goats is different; it is subjected to individual variations and depends on the season. So, for example, in South Korea average duration of spontaneous heat in the local breed of Korean native black goats makes 33.2 hours, with hormonal induced heat it increases to 34.8 hours [4]. The results of our previous researches to determine the time of ovulation in Saanen goats with spontaneous heat have shown that it did not reveal any goat with ovulation before 24 hours from the start of estrus. Most of ovulations (80 %) occurred in the period from 26 till 34 hours from the beginning of estrus, on the average of 28 hours after the onset of heat signs.

In our experiments, on the contrary, a hormonal intervention has significantly reduced the duration of sexual heat, on the average 21.5 hours instead of 32 hours at spontaneous heat during a sexual season (*Мамонтова et al.., 2014*). Accordingly, the time of ovulation can be shifted considerably towards decrease. Therefore, to maximize the fertility of eggs we recommend to carry out artificial

insemination of donors with the induced heat 2-3 times during estrus starting from the 4th hour after coming in heat.

Conclusion

The study results showed highly significant effect of ear implant "Crestar" to prolong the luteal phase of the sexual cycle and synchronization hunting of goats. Hormonal stimulation scheme folliculogenesis, based on the use of drugs "Ovagen", "Folligon" and "Chorulon" the proposed scheme provides optimal ovarian response with an average level of multiple ovulation of 11,4 yellow bodies per animal at average recovery embryos 81,4% and 8,7 suitable for further manipulation of embryos per donors. Proposed scheme is an effective biotechnological technique.

Rezultati indukcije folikulogeneze i proizvodnje embriona sanskih koza

M.I. Selionova, M.M. Aybazov, T.V. Mamontova, M.P. Petrovic

Rezime

U transplantaciji embriona, postoje neke naknadne manipulacije, od kojih je indukcija poliovulacije kod davalaca jedan od najvažnijih elemenata. Pri tome, glavni problem se sastoji u varijabilnosti reakcije jajnika na lečenje. Predloženi plan može da se koristi da podstakne poliovulaciju kod koza donatora za transplantaciju embriona sa visoko sinhronizovanom ovulacijom koja ne zavisi od početnog hormonalnog obrasca donatora. Upotreba ušnog implantata "Crestar" kako bi se produžila lutealna faza seksualnog ciklusa i sinhronizacije je efikasna biotehnološka tehnika. Ona pokazuje da se progestogen iz implantata aktivno izlučuje u krvotok, njegova koncentracija u perifernoj krvi je bila dovoljna za inhibiciju FSH od strane posteriorne hipofize. Hormonska stimulacija šeme folikulogeneze, na osnovu upotrebe preparata "Ovagen", "Folligon" i "Chorulon", predloženi plan obezbeđuje optimalnu reakciju jajnika sa prosečnim nivoom višestruke ovulacije od 11,4 žutih tela po životinji u proseku za procenat dobijanja embriona od 81,4% i 8,7 pogodnih za dalju manipulaciju embriona po donatoru. Treba napomenuti veliku varijabilnost razvojnih faza ovih ćelija. Tako je od procenjenih 96 ćelija, 10 ćelija (9,6%) bilo neoplođeno, 64 ćelije (66,6%) su bile u fazi 2 pronukleusa, 22 ćelije (22,9%) su bile u fazi 2 i 4 blastomere.

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CONSERVATION OF AUTOCHTHONOUS SHEEP BREEDS IN SERBIA AND SLOVENIA

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Abstract: The objective of this paper is to review the status of autochthonous breeds of sheep in Serbia and Slovenia, of their conservation breeding program goals and incentives.The adopted definition of a breed is either a sub-specific group of domesic livestock geographical and/or cultural separation from phenotypically similar groups has led to acceptance of its separate identity. Autochthonous breeds were developed in specific rearing conditions and consequently have specific genes responsible for good adaptability and fertility, disease resistance, maternal instinct and longevity. The autochthonous breeds of sheep in Serbia include: Pirot Pramenka Krivovirska, Bardoka, Karakachanska, Lipska, Vlaskho Vitoroga Pramenka and Chokan Tsigai, whereas in Slovenia: Istria Pramenka, White Landscape Pramenka, Lake-Solčavska and Bovec sheep.The main purpose of conserving the autochthonous sheep breeds in general is to preserve resistance, longevity, maternal instinct, the specificity of each race, increasing the number of heads or maintaining a stable level, as well as preventing of inbreeding. With regard to conservation of genetic resources in Serbia is represented by model in situ, while in Slovenia the *in situ* and *ex situ* program. The incentives in Serbia per head for endangered breeds of sheep is 37 EUR, in Slovenia 13.4 EUR per head.

Key words: sheep, autochthonous, Pramenka, conservation program

Introduction

According to *FAO (1999)* adopted definition, the breed is: »Either a sub-specific group of domestic livestock with definable and identifiable external characteristics that enable it to be separated by visual appraisal from other similarly defined groups within the same species or a group for which geographical and/or

cultural separation from phenotypically similar groups has led to acceptance of its separate identity«. Hall (2004) also compares several proposals for breed definition and prefers the simplest one, given by *Lerner and Donald (1966)*: "a breed is whatever a government says it is". Statement of Keith Hammond's "a breed is a breed if enough people say it is" could probably be overgeneralizing *(Woolliams and Toro, 2007).*

FAO (2007) developed the classification system for breed populations, distinguishing among local breeds and regional or international trans-boundary breeds. Many different terms exists such as “autochthonous”, “native”, “indigenous”, "heritage", "patrimonial", "naturalized" or "locally adapted" which are used in different countries for breed classification. Terms “native”, “indigenous” and “autochthonous” are frequently used and could be synonymous. Autochthonous breeds were developed in specific rearing conditions and consequently have specific genes responsible for good adaptability and fertility, disease resistance, maternal instinct and longevity. Unfortunately, they are losing competition with more productive, intensively reared breeds, where the profit is the primary goal.

Autochthonous breeds, in addition to being a source of genetic diversity for livestock production, constitute a part of the national heritage and provide the identity of the local community. The long-term survival of these breeds is not only a matter of insurance for the future needs of agriculture development. The conservation is a matter of protecting and cherishing cultures and traditions that helps to sustain local communities. In the majority of the European countries, farmers who are keeping endangered autochthonous breeds are supported by the state. In the case of 28 EU Member States incentives are managed by agro-environmental programmes within the National Rural Development Programmes. Nevertheless, levels, scopes of supports, rules applied and practical arrangements differ substantially among the countries *(Subsibreed, 2014)*.

The aim of the study was to collect data about autochthonous sheep breeds in Serbia and Slovenia, and to compare conservation programs, breeding goals and incentives.

Short description of breeds

Autochthonous populations of Pramenka (Zackel) sheep represent a unique genetic inheritance existing thousands of years and as such are an important element of regional agro-biodiversity, tradition and cultural heritage of Serbia and Slovenia as well. Specific climatic, hydrological, feeding conditions and migration caused the formation of different strains (ecotypes) of Pramenka, which differ among each other by morphological, reproductive and productive traits

(Stojanović, 2005; 2006; 2009). Due to significant differences among individual strains in Slovenia, Croatia and Bosnia and Herzegovina, strains acquired the status of the breed.

Short description of autochthonous sheep breeds in Slovenia

In Slovenia four autochthonous sheep breeds are geographically distributed: Istrian Pramenka, Bela Krajina Pramenka, Jezersko-Solčava sheep and Bovec sheep.

The Bela Krajina Pramenka

The breed is widespread in the southeast part of Slovenia and is used for the lamb production (Figure 1). The ewes of the Bela Krajina Pramenka weigh over 50 kg, while rams over 65 to 70 kg. The animals have long fringed wool, which is not appropriate for manufacturing. The coat colour is mainly white with black spots on the head and legs. The sheep's tails are very long almost reaching the ground. Rams have extremely large horns, which are curled several times according to the age. Ewes may have horns as well but shorter *(Kompan et al., 1996)*. Ewes are seasonally poly-estruses with an average 1.18 lambs per year *(Zajc et al., 2015)*. Lambs are reaching 25 kg in their third or fourth month. The Bela Krajina Pramenka lambs have high dressing percentage due to thin bones *(Grabrijan, 1996)*. The population size is 900 animals and according to the national rules the population is endangered *(Register…, 2014)*.

Figure 1: The Bela Krajina Pramenka

The Istrian Pramenka

The Istrian Pramenka is widespread in the region of Karst and Istria and is used for milk production (Figure 2). Rams weight up to 95 kg, while ewes from 60 to 75 kg. The wool is white, black or white with black dots and does not cover the whole body (belly). Horns are present or not. The litter size is 1.05 lambs *(Zajc et al., 2015)* and the sheep produce 119 kg of milk yield in lactation. The milk contains in average 7.2% milk fat and 5.9% milk proteins. The population size is 1.150 animals *(Register…, 2014)* and according to the national rules the population is endangered.

Figure 2: The Istrian Pramenka

The Bovec sheep

The Bovec sheep is widespread in the upper Soča valley and it is used for milk production (Figure 3). The body weight of rams is between 45 and 50 kg, while ewes weight from 35 to 40 kg. The wool is rough, white, brown or black. Sheep are horn-less with short ears. The milk yield is in average 221 kg with 6.3% milk fat and 5.5% milk protein. The population size is 3.400 animals *(Register…, 2014)* and according to national rules the population is at risk.

Figure 3: The Bovec sheep

The Jezersko-Solčava sheep

The Jezersko-Solčava sheep is widespread across Slovenia and it is used for lamb production (Figure 4). The body weight of ewes is between 65 and 75 kg, while rams weigh over 100 kg. The coat colour is mainly white, while there are some sheep with brown colour. Ewes are year-round poly-estruses. The litter size is 1.32 lambs *(Zajc et al., 2015)*. The population size of Jezersko-Solčava sheep is 17.000 and according to national rules population is not endangered *(Register…, 2014)*.

Figure 4: The Jezersko-Solčava sheep

Table 1: The Population size of four Slovenian sheep breeds from 1990 to 2014

Breed/Year	1990*	2000	2010	2014
Jezersko-Solčava sheep	3.000	4.500	17.200	17.000
Bovec sheep	1.200	1.500	3.500	3.400
Bela Krajina Pramenka	400	600	880	900
Istrian Pramenka	200	250	1.150	1.150

*estimation

Short description of autochthonous sheep breeds in Serbia

Autochthonous sheep breeds were described by a large number of authors *(Belić et al. 1986, Mitić, 1986; Petrović and Nenadić, 1992; Radović et al., 1997; Krajinović et al., 1997; Ruzic-Muslić, 2002; 2006; 2007; 2011; 2012; Trailović et al., 2006; Ćinkulov et al., 2008; Savić et al., 2011; Savić et al., 2012; Petrović et al., 2013).* The main reason for decreased population size of different Pramenka breed, in last two decades, was the uncontrolled introgression and displacement crossing with cosmopolitan breeds (Merinolandschaf, Ile de France) for the fattening performance improving. Consequently, certain strains of Pramenka acquire the status "critical" and "vulnerable".

Pirot Pramenka

Pirot Pramenka is a multi-purpose breed (milk, meat, wool) widespread in the Pirot region, in south-eastern Serbia, on the Stara Planina through Pirot, Dimitrovgrad, Babušnica to Vlasina, Leskovac, Bela Palanka and Niš. The body is covered with white wool, except the face and lower parts of legs. The body weight of adult females is 50 - 55 kg, and rams varies in the range of 60 - 70kg. The fertility varies from 105 - 115%. Lambs are born with a body weight of 3 - 4 kg, and achieve a daily gain of 180 - 200 g. Body weight at 90 days of age is 20 - 22 kg, while ewes at 12 months weighing 38 - 45 kg. Milk yield varies from 70 - 100 litters per 180 days of lactation. The wool yield assortment in B, averaging 1.5 - 2.5 kg in ewes and 3 - 4 kg in rams. The Pirot Pramenka is suitable for rearing in all regions and production system *(Petrović et al, 2013)* and is known for its famous products, like Pirot cheese, Pirot lamb and Pirot kilim (wool). The population size is officially estimated on 27 heads *(DAD IS, 2014)*.

Figure 5: The Pirotska Pramenka

Krivovir Pramenka

Krivovir Pramenka is reared in the eastern Serbia, next to the area of Krivi Vir. It is a multi-purpose breed (milk, meat, wool). The body is covered with a white fleece except the face and lower parts of legs. The head and legs are covered with a hair which is a monochrome yellow or scattered yellow colour. The head are long and the fleece on the scalp formed like cap (ćubu). Ewes are hornless while rams have spiral horns. The body weight of adult females is 50 - 55 kg, and rams are in range of 60 - 70 kg. Fertility ranges from 105 - 115%. Lambs are born with a weight of 3 - 4 kg, providing daily gain of 180 – 200 g. In the age of 90 days, they achieve a weight of 20 - 22kg. Milk yield is 30 - 70 litters in 180 days of lactation. The wool yield, assortment B, averages from 1.5 - 2.5 kg in ewes and 2.5 - 3.5 kg in rams *(Petrović et al, 2013)*. The population size is 108 heads *(DAD-IS, 2014)*.

Figure 6: The Krivovirska Pramenka

Bardoka (White Metohian Sheep)

Bardoka is widespread in Metohija and Kosovo as well as in some parts of Montenegro. It's got the name from the white wool. In Albanian language the word "Bardoka" means the white sheep. In Serbia, it is present in the area of Stara Planina and the upland area of Pester. The body is covered with white wool, on the head, legs and ears. This is one of the larger strains of Pramenka. Body weight of ewes ranges from 38 - 45 kg, while rams from 60 - 67 kg. Bardoka is claimed to be the best dairy Pramenka. Ewes produce about 100 litters of milk, including the sucked milk, in 180 days of lactation based on modest rearing conditions. Fertility is about 105%. The wool yield is 1.2 - 2 kg in ewes and 2.5 kg in rams *(Petrović et al, 2013)*. The population size is 36 heads *(DAD IS, 2014)*.

Figure 7: The Bardoka (White Metohian Sheep)

Lipska pramenka

Lipska Pramenka is reared near Smederevo in the village Lipa, after which it was named. The colour of wool is white, while the head and legs are covered with black hair. The average body weight of ewes is about 60 kg, and rams around 65 kg. Lambs birth weight is from 3.75 - 5.0 kg. In 180 days of lactation, ewes produce 100 litters of milk in average. Wool yield, assortment D, in ewes is 1.5 kg and 2.0 kg in rams in average *(Petrović et al, 2013)*. Population size is represented by 513 heads *(DAD IS, 2014)*.

Figure 8: The Lipska Pramenka

Karakachan Pramenka

Karakachan Pramenka is widespread in southeast Serbia, Bulgaria, Macedonia and Greece. A small sheep with strong constitution is covered with black wool except the head, ears and the lower parts of legs that are covered with black hair. The body weight of adult ewes is 35 - 45 kg, and rams varying in the range of 40 - 50 kg. Fertility varies from 105 - 110%. Lambs birth weight is 2 - 3 kg, and at 90 days reached a weight of 15 - 18 kg. Milk yield is poor, ranging from 30 - 40 litters in the 180 days of lactation period. Wool yield, assortment C, is about 2.0 kg in ewes and 3.0 kg in rams *(Petrović et al, 2013)*. The population size is 67 heads *(DAD IS, 2014)*.

Figure 9: The Karakachan Pramenka

Vlaskho Vitoroga Pramenka

Vlaskho Vitoroga Pramenka is grown in the area of South Banat, in Kovin and Deliblato Sands. The fleece colour is white or light brown. The head and legs are covered with white hair. Very special characteristic of breed are large spiral twisted horns. The ewes' average body weight is about 35.0 kg while 40.0 kg in rams. The average milk yield during 100 - 150 days of lactation is 80 - 110 litters. Ewes' fertility is 120% *(Savić et al., 2013)*. The fineness of wool is reached to 38 μm in ewes and 41 μm in rams. The population size is 466 heads *(DAD IS, 2014)*.

Figure 10: The Vlaskho Vitoroga Pramenka

The Chokan Tsigai

The Chokan Tsigai is a lowland multi-purpose sheep breed. The breed originated from the Asia Minor, from where it was spread throughout the Eastern Europe. In the eighteenth century has expanded from Romania to Vojvodina and plain land areas of Serbia. The wool colour is white. The legs and head are covered with black hair. The ewes' body weight is 70 - 75 kg, while rams from 110 - 120 kg. In the intensive fattening conditions, lambs at 90 days achieve 31 kg of body weight. Milk yield is remarkable and ranges from 50 - 150 litters in 180 days of lactation period. Ewes produce in average 2.5 - 4 kg greasy wool, while rams 3.5 - 5.0 kg. The population size is 480 heads *(DAD IS, 2014)*.

Figure 11. The Chokan Tsigai

Table 2. The population size of endangered sheep breeds in Serbia *(DAD - IS,2014)*

Breed/Year	**2005**	**2009**	**2012**	**2013**	**2014**
Pirot Pramenka	450	40	60	66	27*
Krivovir Pramenka	300	300	125	356	108*
Bardoka	900	50	50	47	36*
Lipska Pramenka	70	150	380	470	513*
Karakachan Pramenka	-	-	130	84	67*
Vlach Vitoroga Pramenka	46	350	219	425	416*
Chokan Tsigai	300	450	632	223	480*

* Data refer to animals with a full pedigree; animals with incomplete pedigree were not taken into consideration

There was an evident negative trend of population size of Pirot, Krivovir and Karakachanska Pramenka as well as Bardoka. The encouraging fact is that the population size of Lipska Pramenka, Vlach Vitoroga Pramenka and Chokan Tsigai increased.

Risk status assessment

Risk status assessment for farm animal genetic resources in Slovenia

In Slovenia, the assessment of risk status for farm animal genetic resources is managed by Public service. In accordance with the Rules on biodiversity in livestock (*Ur. L. RS 90/04*) the risk status is estimated once per year. Till 2014, the assessment of the breed risk status was based only on the purebred female population size, registered in the herd book.

From 2015, the risk status is estimated on several parameters. Final assessment is always represented by the worst / lowest risk status. If only one parameter indicates that the breed is highly endangered, it will be classified according to that parameter. In Slovenia, the following parameters are used:

- Reproductive capacity of species. Species are divided on those with large (poultry, pigs, rabbits) and small (equines, cattle, sheep, goats) reproductive capacity. The risk status estimation is based on the combination of the number of pure-bred breeding females and pure-bred breeding males.
- Trend of population size and the percentage of females bred to males of the same breed. The reproductive capacity is considered as well. Populations are divided according to the trend of population size (increasing, stable or decreasing). The risk status estimation is based on the combination of population size and the number of pure-bred breeding males.
- Geographical distribution is measured as a length (km) of the radius of the circular area within which approximately 75 percent of the population lies. The concentration of population in a limited area or in a limited number of flocks / herds can be an important factor in the case of natural catastrophes and diseases.
- Inbreeding rate (ΔF) is estimated based on the numbers of breeding males and females from the pedigree information. The reliability of pedigree-based estimates of inbreeding depends on the number of generations of ancestry recorded. To obtain meaningful estimates, a minimum of five generations is recommended.

Breed population number in the country of origin is a basic factor for the assessment. However, population number at global level must be taken into account. Many breeds which are at risk in the country of origin can be found in other countries as well.

Risk status assessment for farm animal genetic resources in Serbia

In Serbia, the criteria for the risk degree level are based on the classification recommended by the *FAO (1992). Classification was* partly modified in 2007 and it is based on the number of female breeding animals in the population. In the critical category fall breed whose total number of female breeding animals is less than 100, a number equivalent to that of male breeding animals is less than 5 or breed with a total population of slightly over 100 animals, where 80% of heads of purebreds declined. The risk category includes breeds or endangered populations

with the number of female breeding animals between 100 and 1000 or equivalently the number of male breeding animals between 5 and 20, or breed which has slightly over 1000 head, where the participating animals of purebreds about 80% of the population was decline. In sensitive or vulnerable category are breeds with the number of female breeding animals between 1000 and 5000 or equivalently the number of male breeding animals between 20 and 100 or if the total population is slightly over 5,000, where the participating animals of purebreds about 80% of the population was decline. Semi-stable category has breeds with population size between 5.000 and 10.000 female breeding animals or equivalently between 100 and 500 males. Breed or population with the number of female breeding animals of more than 10,000 heads are not threatened, but it should observe.

Taking into consideration the above criteria four Serbian sheep breeds are at risk or in critical category: Pirot, Krivovir, and Karakachan Pramenka as well as Bardoka. In the endangered category following breeds are classified: Lipska and Vlach Vitoroga Pramenka and Chokan Tsigai.

Animal genetic resource conservation

Program for Animal Genetic Resource conservation in Slovenia

In Slovenia, the monitoring of conservation of farm animal genetic resources is managed by the Public service for animal genetic resources conservation under the Department of Animal Science, Biotechnical Faculty, University of Ljubljana. Monitoring started in the year 1990 with inventory of each autochthonous population (breed), its characteristics, history, production systems and knowledge. After years of work on farm animal genetic resources continued within the long term conservation programme supported by the Ministry of Agriculture. Conservation program for farm animals represents long-term process to ensure the protection and conservation of species, breeds and strains in the Republic of Slovenia. The program is harmonised with the Interlaken Declaration and includes direct and indirect monitoring of endangered autochthonous and traditional breeds. The conservation programme includes: inventory of farm animals, monitoring of the risk status, ways of conservation and biological, phenotypic and genetic characterisation. Public Service provides and maintains genetic reserves of species, breeds and strains of farm animals like a minimum number of live breeding animals, semen doses, ova or embryos. ***In situ*** conservation – is managed on the »Ark-farms«. The main purpose of the farm is to keep and maintains the herds, and makes presentations of the endangered breeds to the general public. Ark-farms are farms, centres, business or institutions that maintain the endangered breeds of animals' *in-situ*. ***Ex situ in vitro*** conservation or

cryopreservation represent a permanent storing of animal genetic resources. Stored genetic material facilitates the retrospective genetic studies of populations, isolation of interesting genomic segments, and preservation of breed specific alleles. Moreover, the finest stored genetic material allows the reconstruction of the population in the case of serious threats or even extinction. The depository of tissues includes isolated nucleic acids (DNA, RNA) as well as tissue samples (blood, skin, hair, muscle) of locally adapted breeds (*Public service…, 2014*). Public Service prepares a report of monitored farm animal genetic resource once per year Annual incentives are additionally financial measures to support the conservation of the endangered autochthonous breeds. Despite two breeds of sheep in Slovenia that are highly endangered, the level of subsidies for sheep included in the environmental payments are equal i.e. 13.41 € *(Subsibreed, 2014)*.

Conservation models for autochthonous breeds of sheep in Serbia

In the Republic of Serbia, the only conservation model of genetic resources is *in situ* implemented by the Ministry of Agriculture and Environmental Protection. This model is an active dynamic approach to the protection of breed in its native environment and is acceptable due to lower initial investments, active function in food production (generating revenue) and maintaining the vitality of the population.

Conservation program *in situ* includes:

- monitoring the population size, structure and distribution of breed,
- genetic consolidation and improvement of breed,
- establishing the production parameters of breeds,
- subsidies per head of endangered sheep breeds in the amount of 4.500 dinars,
- optimization of production systems and technologies suitable for genetic resources,
- to raise public awareness and promotion of endangered breeds.

According to the Regulations on incentives for the conservation of animal genetic resources ("RS Official Gazette", No. 83/13), in the field of sheep belongs Pramenka (Pirot, Krivovir, Bardoka, Lipska, Vlach Vitoroga, Karakachan) and Chokan Tsigai.

Breeding program and breeding objectives

Breeding program and breeding objectives for autochthonous sheep breeds in Slovenia

Breeding programmes for sheep are managed by recognised breeding organization like Association of Small Ruminant Breeders in Slovenia. Biotechnical Faculty (Department of Animal Science), Veterinary Faculty, Agriculture Institute and Regional agriculture chambers carries out specific tasks. Within the breeding program, some specific conservation tasks are included. The main breeding goal in the breeding programmes for endangered autochthonous sheep breeds (Istrian pramenka, Bela Krajina pramenka, Bovec sheep) is to preserve type traits specific for each breed, to prevent increasing of inbriding and to increase the population size or to keep it stable. However, the main breeding goal in the breeding programme for Jezersko-Solčava sheep, which is not endangered, is to preserve good fertility and year round poly-estruses as well as to improve conformation and meat quality. Additional breeding goals are to preserve calm temperament, longevity, resistance and adaptability to poor rearing conditions and the ability of grazing. Breeding goals connected with production (Table 3) depends on the risk status of the breed and includes fertility traits (litter size), production traits according to the breed purpose (daily gain, milk yield, fat and protein content) and to increase the frequency of the ARR allele to improve the TSE resistance. Likewise, breeding goal for Jezersko-Solčava and Bovec sheep include the improvement of wool quality (*Program za izvedbo…, 2010*).

Table 3. Breeding goal for Slovenian sheep breeds until the year 2015

Breed	Main breed purpose	Litter size	Daily gain till weaning (g/day)	Milk yield (suckled + milked)	Milk fat/protein content (%)	ARR genotype frequency (%)
Istrian Pramenka	milk	1.23	230	140	7.2/5.8	40
Bovec sheep	milk	1.30	280	240	6.4/5.5	26
Bela Krajina Pramenka	meat	1.20	220	/	/	40
Jezersko Solčava sheep	meat	1.23	240	/	/	28

Breeding program and breeding objectives for autochthonous sheep breeds in Serbia

The main purpose of conserving the autochthonous sheep breeds in general is to preserve resistance, longevity, maternal instinct, the specificity of each race,

increasing the number of heads or maintaining a stable level, as well as preventing of inbreeding. In the next table shows the breeding goal according to breed.

Table 4. Breeding goal for Serbian sheep breeds

Breed	Main breed purpose	Litter size	Daily gain till weaning (g/day)	Milk yield (litters)	Wool yield (kg)	Wool fineness (μ)
Pirot Pramenka	Meat, milk, wool	1.20	220	100	3.0-4.0	35
Krivovir Pramenka		1.20	220	80	2.5 - 3.5	35
Bardoka		1.10	210	110	2.0 - 3.0	45
Lipska Pramenka		1.20	230	110	3.0 - 4.0	35
Karakachan Pramenka		1.10	200	50	2.0 - 3.0	35
Vlach Vitoroga Pramenka		1.15	/	/	2.0 - 3.0	40
Chokan Tsigai		1.30	/	/	2.5 - 4.0	33

In order to managed and improve the future work on conservation of autochthonous sheep breeds in the Republic of Serbia, it is necessary to:

- Human resource development, the process of equipping individuals with the understanding, skills and access to information, knowledge and training that enables them to perform effectively.
- Organizational development, the elaboration of management structures, processes and procedures, not only within organizations but also the management of relationships between the different organizations and sectors (public, private and community).
- Institutional and legal framework development, making legal and regulatory changes to enable organizations, institutions and agencies at all levels and in all sectors to enhance their capacities.
- Monitor and coordinate national legislation with the EU and other international regulations,
  provide material and logistical assistance for the survival and stabilization of vulnerable populations or sheep breeds,
- To promote the sustainable use and proper management of AnGR with an aim of encouraging the multifunctional aspect of agriculture as a whole: valorisation of different forage resources in marginal agricultural areas, the production of safe food, diversification of the rural economy, development of agro-tourism in protected areas,
- Integration of AnGR concept with modern systems of sustainable development (eg, organic, biodynamic) and to undertake popularization of

AnGR by organizing exhibitions and trade fairs of autochthonous sheep breeds

- Provide funding and facilitate the development of investigations relating to genetic characterization of autochthonous sheep breeds,

Conclusion

Conservation of animal genetic resources for food and agriculture, sustainable use and development is responsibility of countries on national and global level. Growing population, changes in consumer demand, climate change and emerging diseases are factors which require adaptability of autochthonous breeds and potential to face an uncertain future. Therefore, we have to make certain that AnGR and especially autochthonous breeds will be conserved for the future generations. Slovenia adopted the Long term Conservation Programme harmonized with the FAO Global Plan of Action and Interlaken Declaration. Consequently, autochthonous sheep breeds are monitored sufficiently and population size remain stable or increased for the last few years.
In Serbia for the future the inventory and characterization of sheep breeds, monitoring of trends and risks are necessary steps for the future work. The additional criteria for the level of the endangerment should be accepted, such as: size of the female population, effective population size, degree of kinship, population trend, the geographic dispersion of population and reproductive ability. In addition to the *in situ* conservation models, the introduction of *ex situ* conservation system for the endangered sheep breeds in Serbia should be start.

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Konzervacija autohtonih rasa ovaca u Srbiji i Sloveniji

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Rezime

Cilj ovog rada je pregled stanja autohtonih rasa ovaca u Srbiji i Sloveniji, njihovih programa očuvanja odgajivačkih ciljeva i podsticaja.

Prema *FAO (1999)* usvojenoj definiciji, rasa je: »Bilo sub-specifičnu grupa domaćih životinja sa definisanim i prepoznatljivim spoljnim karakteristikama koje omogućavaju da se vizuelno razlikuju od drugih slično definisanih grupa u okviru iste vrste ili grupe za koje je geografski i/ili kulturno odvajanje od fenotipski sličnih grupa dovelo do prihvatanja njihovog posebnog identiteta «.

Autohtone rase su razvijeni u specifičnim uslovima gajenja i samim tim imaju specifične gene odgovorne za dobru prilagodljivost i plodnost, otpornost na bolesti, materinski instinkt i dugovečnosti.

U autohtone rase ovaca u Srbiji spadaju: Pirotska pramenka, Krivovirska, Bardoka, Karakačanska, Lipska, Vlaško vitoroga i Čokanska cigaja, a u Sloveniji: Istarska Pramenka, Belo Krajinska Pramenka, Jezersko-Solčavska i Bovška ovca.

Glavni cilj očuvanja autohtonih rasa ovaca uopšte je očuvanje otpornosti, dugovečnosti, materinskog instikta, specifičnosti svake rase, povećanje broja grla ili održavanje stabilnog nivoa, kao i sprečavanje inbridinga.

U pogledu konzervacije genetičkih resursa u Srbiji je zastupljen model *in situ*, dok su u Sloveniji zastupljeni programi *in situ* i *ex situ*.

Podsticaji u Srbiji po grlu za ugrožene rase ovaca iznose 37 EUR, a u Sloveniji 13.4 EUR po grlu.

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OESTRUS SYNCHRONIZATION EFFICIENCY AND FERTILITY IN EWES OF MIS SHEEP POPULATION DURING ANOESTROUS AND BREEDING SEASON

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Abstract: The purpose of this study was to investigate fertility in the ewes of MIS sheep population following synchronized oestrus in anoestrus season and natural oestrus during breeding season. Research was carried out on an experimental sheep farm of the Institute for Animal Husbandry in Belgrade. The study included 76 ewes and 6 rams of MIS sheep population (three breed crosses of Pirot pramenka, Württemberg and Ile de France breed). In anoestrus season, oestrus was synchronized in 43 ewes using progestagen impregnated vaginal sponges (30 mg fluorogestone acetate) in combination with 750 IU PMSG. During the breeding season, 33 ewes were bred naturally to 6 rams, without the use of exogenous hormones. Teaser rams with marking-harnesses and abdominal aprons were used to detect females in oestrus. Obtained fertility and productivity results in anoestrus and breeding season were as follows: lambing rate 69.77% (30/43) and 100% (33/33) ($P<0.01$), gestation length 146.63 and 149.18 days ($P<0.01$), litter size 2.33 and 1.81 ($P<0.05$), number of stillborn lambs/ewe 0.47 and 0.06, overall number of stillborn lambs 15 and 2 ($P<0.05$), birth weights of lambs 3.40 and 4.64 kg ($P<0.01$), body weights of lambs at 30 days of age 10.71 and 12.35 kg ($P<0.01$), litter weight at birth 6.24 and 8.13 kg ($P<0.05$) and litter weight at 30 days of age 16.99 and 20.32 kg ($P<0.05$).

Key words: oestrus synchronization, natural oestrus, fertility, reproduction, MIS sheep

Introduction

Reproduction, as a fundamental link in the conservation of species, is considered the most vital function of living organisms. In sheep production,

success is estimated by a number of offspring per sheep and year. However, sheep exhibit seasonal reproductive activity, with a break in the reproduction during the long spring days and activation of reproductive activity with the advent of fall short photoperiod *(Dogan and Nur, 2006)*. The breeding season of sheep in Serbia starts from the mid-summer until the end of fall/beginning of winter. Thus, reproductive seasonality is an important factor that limits the productivity of small ruminants *(Zarazaga et al., 2003)*. For this reason, hormonal induction/synchronization of oestrus in sheep is used as a means for improving reproductive efficiency of the flock. In addition, the use of oestrus synchronization creates the opportunity for timed breeding and lambing *(Abdalla et al., 2014)*.

In small ruminant, oestrus synchronization is achieved either by reducing the length of luteal phase of the oestrus cycle with prostaglandin F2α or its analogues or by extending the cycle artificially with exogenous progesterone or more potent progestagens *(Jainudeen et al., 2000)*. Progestagens are widely used to synchronize estrus in sheep and typically result in greater than 90% of ewes in heat in a 24-hour period and conception rate of 70–80% *(Evans et al., 2001)*. Most widely used procedure for estrus induction/synchronization is application of progestagens through vaginal sponges in combination with PMSG or FSH, especially outside the breeding season.

MIS sheep population is a meat type of sheep, of strong constitution, strong carcass conformation and good meat properties. It was obtained by using a complex combination crossing according to a precisely defined genetic procedure, using Pirot Pramenka, Wuerttemberg and Ile de France breeds *(Petrović, 2006)*. Ewes of MIS sheep population reach sexual maturity at age of 6-8 months, which puts them in the group of fast maturing populations. According to *Petrović (2006)* ewes of MIS population have an average fertility of 130 to 160%.

However, there is not enough information in the literature regarding synchronization efficiency and fertility in ewes of MIS sheep population during breeding and anoestrus season. Good reproductive performance is a prerequisite for any successful production. Low reproduction means low production of meat or milk. For this reason it is important to determine the actual level of reproductive performance in every flock. Thus, the purpose of this study was to investigate fertility in the ewes of MIS sheep population following synchronized oestrus in anoestrus season and natural oestrus during breeding season.

Material and methods

Location of the experiment and animals

Research was carried out on an experimental sheep farm of the Institute for Animal Husbandry in Belgrade. The study included 76 ewes and 6 rams of MIS sheep population. Ewes were 2 to 5 years of age, and rams were 13 and 18 months old. Previous to mating, rams were kept separately from females. Both rams and ewes were kept outdoors at pasture.

Experimental design

Off-season oestrus (during the month of April) was synchronized in 43 ewes using progestagen impregnated vaginal sponges (30 mg fluorogestone acetate, FGA, Syncro-Part, Ceva sante animale, France) in combination with PMSG. Sponges remained *in situ* for 12 days. On the day of sponge removal, ewes received an intramuscular injection of 750 IU PMSG. Six rams were introduced to ewes 48 hours later and were kept with them for mating for about 8-10 hours. Ram to ewe ratio for mating was 1:7-8 (1:1-2/day).

Within the breeding season, 33 ewes were bred naturally to 6 rams, without the use of exogenous hormones, from mid-August to mid-October. Teaser rams with marking-harnesses and abdominal aprons were used to detect females in oestrus. Marked ewes were allocated from the flock and joined with breeding rams in individual pens. Rams remained with ewes for approximately 8 to 10 hours during mating. Ram to ewe ratio for mating was 1:5-6 (1:1-2/day).

The measured traits

The following reproductive parameters were measured: lambing rate (number of ewes lambed/number of ewes mated), gestation length, litter size (number of lambs born/number of ewes lambed), the number of stillborn lambs per ewe and overall number of stillborn lambs, birth weight of lambs, body mass of lambs at 30 days of age, litter weight at birth and litter weight at 30 days of age.

Statistical analysis

Statistical analysis of the experimental data was performed using the statistical package Statistica for Windows 7 (Stat. Soft. Inc.). The equality of variances of analyzed treatments was tested using the Leven's test. Lambing rates and number of stillborn lambs were tested by Chi square analysis. Gestation length, litter size, birth weight of lambs, body mass of lambs at 30 days of age, litter weight at birth and litter weight at 30 days of age were analyzed by one-way analysis of variance. Analyses were performed for the significance level of 5% and 1%.

Results and Discussion

Table 1 shows the average values of investigated reproductive and productive traits, obtained after the off-season oestrus synchronization and natural seasonal mating in ewes of MIS sheep population.

Table 1. The mean (±se) fertility results after off-season oestrus synchronization and natural seasonal mating

Parameters	Off-season synchronized oestrus	Seasonal mating
Lambing rate (%)**	69.77±5.95	100±0.00
Gestation length (days)**	146.63±0.44	149.18±0.19
Litter size*	2.33±0.19	1.81±0.08
Number of stillborn lambs/ewe	0.47±0.19	0.06±0.04
Number of stillborn lambs* (sum)	15	2

*P<0.05 **P<0.01

Mean values for birth weight of lambs, body weight of lambs at the age of 30 days, litter weight at birth and litter weight at 30 days of age, obtained after the off-season oestrus synchronization and natural seasonal mating, are set out in Table 2.

Table 2. The mean (±se) results for some productive traits after off-season oestrus synchronization and natural seasonal mating

Parameters	Off-season synchronized oestrus	Seasonal mating
Birth weight of lambs (kg)**	3.40±0.12	4.64±0.11
Body mass of lambs at 30 days of age (kg)**	10.71±0.33	12.35±0.37
Litter weight at birth (kg)*	6.24±0.61	8.13±0.39
Litter weight at 30 days of age (kg)*	16.99±0.98	20.32±1.15

*P<0.05 **P<0.01

As shown in Table 1, lambing rates were 69.77% (30/43) for off-season synchronized oestrus and 100% (33/33) for natural seasonal mating, which was significantly different at P<0.01. These results, which demonstrate a lower conception and lambing rates in sheep after the hormonal induction and synchronization of oestrus outside the breeding season, are common and consistent with studies of other authors *(Dogan and Nur, 2006; Santos et al., 2011; Moradi Kor et al., 2012). Scaramuzzi et al. (1988)* and *Gottfredson (2001)* stated that

fertility is often reduced after hormonal induction and synchronization of oestrus, due to the lower conception in ewes, which is caused by insufficient synchronization of oestrus and ovulation. *Evans et al. (2001)* stated that use of progestagen for oestrus synchronization typically results in greater than 90% of ewes in heat in a 24-hour period and conception rate of 70–80%.

Average gestation length was found to be 146.63 days (range 141-152) for off-season synchronized oestrus and 149.18 days (range 146-151) for natural seasonal mating, which was significantly different at $P<0.01$. In support of these results are investigations of *Safranski et al. (1992)* and *Horoz et al. (2003)* in which was stated that hormonal treatment of induction and synchronization of the reproductive cycle of sheep shortens gestation length. *Timurkan and Yildiz (2005)* have established a difference in gestation length of 11 days, between hormonally treated and control ewes, but also within the group of treated ewes concerning a dose of applied PMSG. They found that higher doses of PMSG shorten period of gestation and so, the explanation for the differences in the gestation length can be found in the application of PMSG.

Beside lambing rate, litter size, expressed as number of born lambs per ewe lambed, is considered as very important factor of sheep fertility and productivity. In the present study, average litter size of ewes bred after off-season synchronized oestrus was 2.33 (range 1-5), which was significantly different ($P<0.05$) from average litter size of 1.81 (range 1-3) found in ewes bred during natural mating season. Average litter size in ewes of MIS sheep population found by *Petrović (2006)* was 1.3 to 1.6, which is lower than the values recorded in the present study. With hormonal manipulation of sexual cycle in sheep, especially with the application of PMSG or FSH, there is a greater chance for intense ovarian activity and ovulation of greater number of oocytes that will potentially be able to become fertilized, which is basically the same principle that applies in embryo transfer programs for induction of multiple ovulation. This is in agreement with findings of *Safdarian et al. (2006), Koyuncu and Altıcekic (2010), Thammakarn (2011)* and *Ince and Koker (2011)*.

Number of stillborn lambs differed significantly ($P<0.05$) in the two studied periods, 15 in off-seasonal and 2 in seasonal mating, or an average of 0.47 per ewe in off-seasonal and 0.06 in seasonal mating. A greater number of lambs per litter and lower birth weights have contributed to the greater number of stillbirths after off-seasonal fertilization. Previous investigations by *Purser and Young (1964), Wiener et al. (1983)* and *Berger (1997)* confirm the findings of a higher rate of stillbirth in numerous litters. *Berger (1997)* also stated that highly prolific sheep breeds normally have higher mortality rates of lambs at birth compared to less fertile breeds, because of the more numerous litters.

Birth weights and body weights of lambs at 30 days of age were found to be significantly different (P<0.01) between lambs born from off-season synchronized oestrus (3.40 and 10.71 kg) and natural mating season (4.64 and 12.35 kg). These values are in accordance with results obtained by *Petrović et al. (2009a)*. A greater number of lambs born per ewe in off-seasonal mating caused lower body weights of lambs at birth, which is the difference that held until the first 30 days of life of lambs. As a result, the litter weight, both at birth and at the age of 30 days, was higher after seasonal mating, which was significant at (P<0.05). Type of birth is one of the well-documented factors that have a significant impact on birth weight of lambs and it is expected that the increase of the number of lambs in the litter reduce their body weight *(Fourie and Heydenrych, 1982; Mekić et al., 2004; Petrovic et al., 2009b; Yilmaz and Atin, 2011)*. Also, ewes which carry multiples and their lambs are more likely to be exposed to the risk of insufficient nutrition before and after parturition, unless the diet is based on litter size and for every ewe in particular.

Conclusion

Reproductively, MIS sheep population is very good if well managed. Results of this study suggest that fertility in the ewes of MIS sheep population, bred naturally inside breeding season, is very good, with high lambing and fecundity rates. As for synchronized off-seasonal oestrus, administration of progestogens (FGA), using intravaginal sponges, and PMSG to MIS sheep ewes appear to be effective in the induction/synchronization of oestrus and in increasing of mean litter size. However, numerous litters and lower birth weights of lambs led to higher stillborn rates and lower weaning weights. In this respect, we suggest that in further investigations lower doses of PMSG are applied to avoid effect of superovulation.

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Efekat sinhronizacije estrusa i plodnost ovaca MIS populacije u periodu anestrije i prirodne sezone parenja

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Rezime

Cilj ovog istraživanja bio je da se ispita plodnost ovaca MIS populacije tokom prirodne sezone parenja kao i nakon hormonski indukovasnog/sinhronizovanog estrusa tokom perioda anestrije. Istraživanje je sprovedeno na eksperimentalnoj farma ovaca Instituta za stočarstvo u Beogradu. U ogled je bilo uključeno 76 ovaca i 6 ovnova MIS populacije (trorasni melez dobijen kombinacijskim ukrštanjem sledećih rasa: Pirotska pramenka, Virtemberg i Il de Frans). Tokom perioda anestrije (april mesec), estrus je indukovan/sinhronizovan kod 43 plotkinje upotrebom vaginalnih sunđera impregniranih fluorogeston acetatom u količini od 30 mg uz kombinovanu aplikaciju SŽK u dozi od 750 ij. Tokom prirodne sezone parenja (avgust-oktobar) 33 plotkinje su prirodno ušle u estrus i parene, bez upotrebe egzogenih hormona. Za otkrivanje plotkinja u estrusu korišćeni su ovnovi probači. Utvrđeni su sledeći reproduktivni i proizvodni rezultati nakon vansezonskog i sezonskog pripusta: stopa jagnjenja 69,77% (30/43) i 100% (33/33) ($P<0,01$), dužina bremenitosti 146,63 i 149,18 dana ($P<0,01$), veličina legla 2,33 i 1,81 ($P<0,05$), broj mrtvorođene jagnjadi po ovci 0,47 i 0,06, ukupan broj mrtvorođene jagnjadi 15 i 2 ($P<0,05$), telesne mase jagnjadi na rođenju 3,40 i 4,64 kg ($P<0,01$), telesne mase jagnjadi u uzrastu od 30 dana 10,71 and 12,35 kg ($P<0,01$), masa legla na rođenju 6,24 i 8,13 kg ($P<0,05$) i masa legla u uzrastu jagnjadi od 30 dana 16,99 i 20,32 kg ($P<0,05$). Rezultati ovog istraživanja pokazuju da je plodnost ovaca MIS populacije veoma dobra, sa visokom stopom jagnjanja i dobrim indexom jagnjenja. Upotreba fluorogeston acetata u kombinaciji sa SŽK je efektivna u indukciji/sinhronizaciji estrusa tokom perioda anestrije. Međutim, brojnija legla iz sinhronizovanih estrusa i niže porođajne mase jagnjadi uslovljavaju i veću stopu mrtvorođenja i niže mase jagnjadi na odlučenju. U skladu sa tim, u narednim istraživanjima trebalo bi ispitati mogućnost upotrebe manih doza hormona kako bi se izbegao efekat superovulacije.

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ASSOCIATION BETWEEN BODY WEIGHT AND SOME MORPHOMETRIC MEASUREMENT OF MATERNAL MIS SHEEP

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Abstract: Animal live body weight (BW), body measurements, and their inter-relationship and correlation is imperative for determining genetic potential. In some circumstances in the absence of weighing scales, the body measurements has been used to predicts the live body weight of animals which at the same time is exclusively important to make the right decision at the selection of sheep.The aim of the study was to assess body measurement and the correlation between live body weight and the morphometric measurements of Mis maternal ewes as well as to determine the best fitted regression model for predicting its live weight. A positive correlation between body measurements of Mis maternal ewes ranged from very weak 0.035 (RH-PBW) to high 0.930 (HAW-RH), while a very weak negative correlation ranged from -.016 (HAW-GSB) to -.088 (GSB-RH). With regards to the correlations between body weights and some morphometric measurements showed that the highest correlation was between BW and HG which is 0.853 while the lowest correlation was 0.145 between BW and RH. The multiple regression coefficient in any of the models statistically significant ($P<0.01$) and explicitly denotes that the regression models significantly predicts the value of the criterion variables.

Keywords: maternal ewes, body weight, morphometric measurement, correlation, linear regression

Introduction

Body measurements supplemented to body weight describes more completely an individual or population than do the conventional methods of weighing and grading (*Ravimurugan et al., 2013)*. Good husbandry practices require that a number of

decisions based on the live weight of animals (*Sackey et al., 2013).* Determining animal live body weight (BW), linear body measurements, and their inter-relationship and correlation is imperative for determining genetic potential, breed standards, and improved breeding programs for higher meat production. (*Younas,et al.,2013).* Several authors have been used body measurement to predict body weights of different sheep breeds *(Atta and El Khidir 2004; Riva et al., 2004; Topal and Macit 2004; Afoloyan et al., 2006; Sowande and Sobola 2007; Tariq et al., 2012).* Live weight plays an important role in determining several characteristics of the farm animals especially the ones having economical importance. Body measurements differ according to the factors such as breed, gender, yield type and age. The live weight estimations using the body measurements is a matter of concern for sheep industry (*Pesmen and Yardimci 2008).* Knowledge of live weight can influence the bargaining of the producer and further ensure fair determination of price for marketed animals rather than subjective visual appraisal method. However, this fundamental knowledge of body weight estimation is often unavailable to farmers due to unavailability of scales. Hence, the farmers have to rely on questionable estimates of the body of their animals leading to inaccuracies in decision-making and husbandry *(Moaeen-ud-Din et al., 2006*; *Mahmud et al., 2014*). The usefulness of correlation analysis in life sciences is enhanced when the coefficient is partitioned into direct effects of one trait on the other and indirect effects caused by other characters which may be of importance in selection *(Yunusa et al., 2014).* The objective of the present study was to evaluate body measurement and to assess the correlation between live body weight and the morphometric measurements of Mis maternal ewes as well as to determine the best fitted regression model for predicting its live weight.

Material and Method

In the study involved 60 Mis maternal ewes' ages 3-4 years at the experimental farm of the Institute for Animal Husbandry. After shearing and 3 months after lambing, the animals had measured by using and an aluminum measuring stick for the height measurements while for the length and circumference had done using a flexible tape. The animals also weighed thru manual sheep weighing crate scale. The data considered in the study were the live body weight and morphometric measurement as the following: Height at wither (HAW) as the distance between the foot of the forelimb to the wither point; Rump height (RH) measured from the hind limb foot to the top of the rump; Body length (BL**)** measured from the point of the shoulders to the pin bones; Pin bone width (PBW) is the distance between the outer edges of the major hip bones on the right and left side; Fore cannon length

(FCL) on the lower part of the leg extending from the hock to the fetlock in hoofed; Girth of shin bone (GSB) – measured round the shin; Heart girth or Girth of chest (HG/GC) measured round the chest immediately behind the forelimbs. The analysis of data performed by using the correlation and regression procedure of the statistical software package SPSS version 20 (2011).

Result and Discussion

The average body weight and averages of some morphometric measurements of Mis maternal sheep displayed in table 1 as follows: BW-69.09 kg; HAW-70.24cm; RH-71.06cm; BL-71.87cm; HW-26.18cm; FCBL-14.74cm; GSB-9.51cm; HG 100.83cm. There are variations in all traits, but highest in BW, and the lowest variations found in GSB.

Table 1. Mean, and standard error (S.E.) of Mis maternal body weight and body measurement

Traits	Minimum	Maximum	Mean		Std. Deviation
	Statistic	Statistic	Statistic	Std. Error	Statistic
BW, kg	49.00	87.00	69.09	1.25	9.71
HAW, cm	63.00	85.00	70.24	.49	3.86
RH, cm	64.00	86.00	71.06	.51	3.98
BL, cm	63.00	79.00	71.87	.43	3.35
HW, cm	23.00	29.00	26.18	.14	1.11
FCBL, cm	13.50	16.00	14.74	.09	.71
GSB, cm	8.00	11.00	9.51	.09	.70
HG, cm	92.00	113.00	100.83	.67	5.22

Table 2. Correlation among the body measurement of maternal Mis sheep

Traits		BW	HAW	RH	BL	PBW	FCBL	GSB	HG/GC
Body Weight	Pearson Correlation	1	.234	.145	.618**	.690**	.347**	.657**	.853**
	Sig. (2-tailed)		.072	.268	.000	.000	.007	.000	.000
Height at Withers	Pearson Correlation	.234	1	.930**	.187	.090	.398**	-.016	.261*
	Sig. (2-tailed)	.072		.000	.152	.494	.002	.902	.044
Rump height	Pearson Correlation	.145	.930**	1	.235	.035	.392**	-.088	.181
	Sig. (2-tailed)	.268	.000		.071	.789	.002	.502	.167
Body length	Pearson Correlation	.618**	.187	.235	1	.417**	.128	.309*	.544**
	Sig. (2-tailed)	.000	.152	.071		.001	.328	.016	.000
Pin bone width	Pearson Correlation	.690**	.090	.035	.417**	1	.292*	.473**	.596**
	Sig. (2-tailed)	.000	.494	.789	.001		.024	.000	.000
Fore cannon bone length	Pearson Correlation	.347**	.398**	.392**	.128	.292*	1	.308*	.384**
	Sig. (2-tailed)	.007	.002	.002	.328	.024		.017	.002
Girth of shin bone	Pearson Correlation	.657**	-.016	-.088	.309*	.473**	.308*	1	.602**
	Sig. (2-tailed)	.000	.902	.502	.016	.000	.017		.000
Heart/Chest girth	Pearson Correlation	.853**	.261*	.181	.544**	.596**	.384**	.602**	1
	Sig. (2-tailed)	.000	.044	.167	.000	.000	.002	.000	

** P<0.01.

*P<0.05

BW-body weight; HAW-height at withers; RH-rump height; BL-body length; PBW-pin bone width; FCL-fore cannon length; GSB-girth of shin bone; HG-heart girth

The correlation between body weight on body measurements and the correlation of among traits are presented. The obtained results (table 2), showed a very significant correlation (P<0.01) between BW-BL, BW-PBW, BW-FCBL, BW-GSB, BW-HG. Likewise between HAW-RH, HAW-FCL, RH-FCL, BL-PBW, BL-HG, PBW-GSB, PBW-HG, FCL-HG, HG-GSB while a significant

correlation acquired between HAW-HG, BL- GSB, PBW-FCL, GSB-BL and GSB-FCL. Among the body measurements, the highest correlation was between RH-HAW with a value of 0.930 and the lowest between HAW-GSB with a negative correlation of -.016. A positive correlation between body measurements of Mis maternal ewes ranged from very weak 0.035 (RH-PBW) to high 0.930 (HAW-RH), while a very weak negative correlation ranged from -.016 (HAW-GSB) to -.088 (GSB-RH). In the result obtained by *Petrovic et al., (2012),* the correlation between body measures of dams Merinolandschaff had a high correlation of 0.999 on BL-GC. The result we acquired in this study for Mis maternal ewes showed a medium correlation of 0.544 on BL-GC. As pointed by *Pesmen and Yaedimci, (2008),* "the body measurement differs by breed" rationalized our results.

With regards to the correlations between body weights and some morphometric measurements, it showed that the highest correlation was between BW and HG which is 0.853 while the lowest correlation was 0.145 between BW and RH. The result of *Yunusa et al., (2014),* (for West African Dwarf sheep) revealed that high correlations with BW, and their indirect effects mostly obtained through HG was agreeable with the result we attained in this study. *Mohammad et al., (2012)* also detected a highly correlation between body weight and chest girth (0.742) and body weight and body length (0.457) on five indigenous sheep breeds (Mengali, Balochi, Harnai, Beverigh and Rakhshani). In the study performed by *Mahmud et al., (2014)* informed that CBL significantly affects LBW of Nigerian breeds of sheep (ages 3 years and above) alike with the result we obtained in maternal Mis Sheep (BW-FCBL; P<0.01). *Otoikhian et al., (2008),* documented that there is a close relationship between body weight and chest girth, which is relevant with the result we obtained.

Table 3. Regression Model Summary

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.853[a]	.727	.722	5.11564	.727	154.403	1	58	.000
2	.882[b]	.778	.771	4.64830	.051	13.249	1	57	.001
3	.896[c]	.802	.792	4.42964	.024	6.766	1	56	.012
4	.908[d]	.825	.812	4.20654	.023	7.097	1	55	.010
a. Predictors: (Constant), HG									
b. Predictors: (Constant), HG, PBW									
c. Predictors: (Constant), HG, PBW, BL									
d. Predictors: (Constant), HG, PBW, BL, GSB									
e. Dependent Variable: BW									

The “stepwise” method has formed models in four steps (table 3). In model 1, it showed a coefficient of multiple regressions (R) of 0.853 as the measure of correlation between the values of BW and the predictor HG, with a coefficient of multiple determination (R2) of 0.727, this means that 72,7% of the variance BW, determined variance of the predictor in model 1, in same manner the adjusted coefficient of multiple determination (adjusted R2) was 0.722 or 72.2% of the variance BW determined variance of the predictor variable that was in model 1. This means that the HG/chest girth is the best predictor in estimating the body weight of Mis maternal sheep.

Viewing of the other model had similarity in scheme so we will proceed directly on model 4 as the final model that shows the highest in coefficient of multiple regressions (R) 0.908. The said value is the measure of correlation between the values of body weights as the dependent variable and the set of predictors (HG, PBW, BL and GSB) that are in the final model. As presented (table 3), the coefficient of multiple determination (R2) was 0.825 meaning 82.5% of the variance BW, determined variance of the predictors represented in the model. Furthermore, it also presented the adjusted coefficient of multiple determinations (adjusted R2) with a value of 0.812 or 81.2% of the variance BW, determined variance of the predictor variables that were in the model. The result attained in this study fitting with the statement of *Ravimurugan et al, (2013)* that the chest girth alone or combinations of three measurements may be used for predicting the body weight (Kilakarsal sheep). *Sackey et al, (2013),* found that HG and BL satisfactorily predicted live body weight of Djallonké ewes by its coefficient of determination (R2) of 94%, and 80% respectively for models fitted for Djallonké ewes. *Topal and Macit, (2004),* commented that the model including heart girth (Morkaraman Sheep) was the best fitted regression model.

Table 4. Results of Analysis of variance

Model		Sum of Squares	df	Mean Square	F	Sig.
1	Regression	4040.682	1	4040.682	154.403	.000[b]
	Residual	1517.844	58	26.170		
	Total	5558.526	59			
2	Regression	4326.943	2	2163.471	100.130	.000[c]
	Residual	1231.583	57	21.607		
	Total	5558.526	59			
3	Regression	4459.712	3	1486.571	75.762	.000[d]
	Residual	1098.814	56	19.622		
	Total	5558.526	59			
4	Regression	4585.300	4	1146.325	64.782	.000[e]
	Residual	973.226	55	17.695		
	Total	5558.526	59			

a. Dependent Variable: BW

b. Predictors: (Constant), HG

c. Predictors: (Constant), HG, PBW

d. Predictors: (Constant), HG, PBW, BL

e. Predictors: (Constant), HG, PBW, BL, GSB

As seen in table 4, the values of F-test in models 1 to 4, showing the values of 154.403 (P=0.000); 100.130 (P=0.000); 75.762 (P=0.000); 64.782 (P0=.000), thus confirming that the multiple correlation coefficient in any of the models statistically significant and explicitly denotes that the regression models significantly predicts the value of the criterion variables.

Table 5. Regression Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.
		B	Std. Error	Beta		
1	(Constant)	-90.757	12.881		-7.046	.000
	HG	1.585	.128	.853	12.426	.000
2	(Constant)	-123.320	14.732		-8.371	.000
	HG	1.272	.144	.684	8.813	.000
	PBW	2.449	.673	.283	3.640	.001
3	(Constant)	-139.901	15.418		-9.074	.000
	HG	1.114	.150	.599	7.407	.000
	PBW	2.216	.648	.256	3.423	.001
	BL	.537	.207	.186	2.601	.012
4	(Constant)	-140.369	14.643		-9.586	.000
	HG	.930	.159	.500	5.856	.000
	PBW	1.908	.626	.220	3.050	.004
	BL	.565	.196	.195	2.875	.006
	GSB	2.646	.993	.192	2.664	.010
a. Dependent Variable: BW						

The four models presented (table 5) can visualize that aside from the regression constant also included predictors in every model. It implies that any increase of the indicated body measurement of maternal ewes is associated with an increase of the dependent variable BW. In particular using model 4, any increase in HG to 1 cm, is associated with an increase in BW to .930 kg. An increase in PBW for 1 cm is associated with an increased in BW for 1.908 kg. Likewise, an increase of 1 cm on BL affiliated with an increase in BW for 0.565 kg. The increase of GSB for 1 cm linked an increase of BW for 2.646 kg. The standardized coefficients (Table 5) specify the size of the standard deviation of changes in BW if value of predictors increased by 1 standard deviation. The situation comply on the statement of *Seifemichael et al., (2014*), that as a criterion, the value of R2 always increased when more and more predictors added to the regression. Based

on the results of the multiple regressions, it showed that a highly significant correlation (P<0.01) between BW from HG, PBW, BL and GSB of Mis maternal ewes. Although *Mohammad et al., (2012)* used Regression Tree Method to predict body weight from body length, body weight from chest girth of yearling sheep also achieved highly significant correlation (P<0.01) on Balochian indigenous sheep breeds.

Conclusion

The result attained determined that body measurement such as heart girt or girth of chest (HG/CG), hip wip (HW), girth of shin bone (GSB), body length (BL) and fore cannon bone length (FCBL) had positive correlation with body weight of Mis maternal sheep (r=0.853; r=0.690; r= 0.657; r=0.618; r=0.357). The highest correlation among morphometric measurements exhibited between rump height and height at Withers (RH-HAW) (r= 0.930) and the lowest between height at withers and girth of shin bone (HAW-GSB) with a negative correlation of (r=-.016). Although the fore cannon bone length had significant correlation on body weight, it was not included as one of the predictors. The simplest model (one predictor) has an R2 value of 0.727, while the full model (all the predictors) has a coefficient determination (R2) value of 0.825. The result obtained indicated that in any increase of some of the body measurements (HG, PBW, BL and GSB) of maternal ewes is also an increase in body weight of maternal ewes. Based on the results acquired on this study it seems that heart girth (HG) alone can be the best fitted predictor of body weight of Mis maternal ewes.

Povezanost između mase tela i nekih morfometrijskih mera kod majki ovaca Mis rase

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Rezime

Masa tela ovaca (BW), morfometrijske mere tela, njihov odnos i korelacija su imperativ za utvrđivanje ekspresije genetskog potencijala. U nekim okolnostima u odsustvu vage , merenje tela se koristi za predviđanje telesne mase životinja koja u isto vreme je i iskljusivo vazna za donosenje prave odluke pri selekciju ovaca. Cilj

istraživanja je bio da se procene vrednosti telesnih mera i korelacije između žive telesne mase i morfometrijskih merenja kod majki Mis ovaca, kao i da se odredi najbolje prilagođen model regresije za predviđanje mase tela ovaca. Pozitivna korelacija između telesnih mera Mis majki ovaca varira u rasponu od veoma slabe 0.035 (RH - PBW) do visoke 0.930 (HAW- RH) , dok je vrlo slaba negativna korelacija evidentirana u rasponu od -.016 (HAW- GSB) do -.088 (GSB - RH). Kada je reč o korelaciji između telesne mase i nekih morfometrijskih merenja pokazalo se da je najveća povezanost između BW i HG sa vrednošću od 0.853 , dok je najniža korelacija zabeležena između BW i RH i bila je 0.145. Koeficijent multiple regresije je bio kod svih modela statistički značajan ($P<0.01$) i eksplicitno označava da regresija značajno predviđa vrednost kriterijumskih varijabli ($P=0.000$)

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MORPHOMETRIC CHARACTERIZATION OF THE DOMESTIC HILLY HORSE POPULATION IN MONTENEGRO

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Invited paper

Abstract: Rearing and use of the horses in long period of the past has had a very big importance for agriculture generally and for the rural society in Montenegro. Horses were the most important work animals for different uses, constantly present in everyday's peasant life and important part of the cultural heritage of the rural areas in Montenegro.
The aim of this paper is to present system of rearing and use, and basic morphometric characterization of Domestic hilly horse population in Montenegro.
The total horse population has been drastically reduced, especially after 60's of last century: from 32,000 animals in 1960 to only 4850 animals in 2013.
Based on the results of this research the most often coat colour in the studied population was bay (48.03%), white marks on head was determined in a half of the population and the most presented marks was faint (22.23%) and white marker on the leg was pastern and combination of more marks. Montenegrin domestic hilly horse belongs to the group of small horses, with average height to withers of 134.2 cm, body length 139.3 cm, chest circumference 163.5 cm, fore cannon circumference 18.67 cm, while body weight was 314.8 kg. The values of indices of body conformation showed that studied horse population had quite strong and compact body conformation and very well work abilities.

Key words: hilly horse, exterior, body measures, indices.

Introduction

The horses and horse breeding, as a branch of animal husbandry, had an important role in a man's life in the past, especially if we take into account multiple uses of the horses. The role and contribution of horses in historical, cultural and economic development have been changing in accordance to the social needs and circumstances (*Ivanković 2004*). In the earliest history horse had strategic importance in wars, and later in transportation of various goods over long distances

and maintains trade links. In the overall use of horses, a special place belonged to its use in agricultural activities and the exploitation of wood. In recent times, new forms of use of horses have been emerging, which are manifested through the sport and recreation. In certain countries or regions of the world the importance of the horse is even in production of meat and milk (*Pejić 1996*).

Horse in Montenegro has always been a very important and indispensable working animal. Without the horse help peasants life in the past was difficult to imagine. In addition to the basic purpose to perform many tasks in agriculture, the horse was an integral part of everyday's life and living culture of the Montenegrin population. Until starting to use mechanization widely and introduction of new technologies in agriculture, horse was probably more than anywhere else one of the main preconditions for the existence of the Montenegrin population (*Adžić 1997*).

The specific natural conditions in Montenegro has shaped and adapted the populations of domestic hilly horse, which has been reared for centuries in Montenegro and it is a segment of the wider population of the Balkan hilly horse. It originates from the original wild form and the Asian wild horse, known as Przhevalskii (*Equus Przewalskii Poliakov*) and the steppe horse Tarpan (*Equus Przevlskii Gmelini Antonius*), *Hrasnica (1962), Pejić (1996).* Thanks to the very good adaptability to specific natural conditions, strong constitution and disease resistance, modesty in regard to nutrition and extraordinary working abilities, Domestic hilly horse was used primarily for carrying and riding (*Paprika and Ivanković 2005, Mitrović and Grubić 2003*)

Numerous studies of exterior and working abilities of mountain horses were carried out in the past, especially in 50's and 60's of the 20th century, mainly on the population of the Bosnian mountain horse (*Malecki, 1923; Ogrizek and Hrasnica, 1952; Belić, 1940; Bartolović 1961; Telalbašić et al. 1973; Habe and Telalbašić, 1988; and Ivankovic et al., 2012*).

Given the fact that the population of Domestic hilly horse has been raised for the centuries in Montenegro, and that has not yet been the subject of significant research, the aim of this work is to present the current state and specificities of horse breeding, as well as the morphometric characterization of Domestic hilly horse population in Montenegro.

Material and methods

The study was conducted during the period 2012-2013 on the population of Domestic hilly horse (118 animals) which is bred in the municipalities: Niksic, Cetinje, Plužine and Šavnik. The first step in the research was to select animals that by general breed characteristics belong to the domestic hilly horse; the second was to analyse current way of breeding and use of horses. Further, the colour of hair and present white characters on the head and legs were determined by the visual

observation. Identification was made using the generally adopted classification of colours and special characters on the head and legs of a horse, described by *Nikolić and Simović (1986)* and *Ivankovic (2004)*.
Determination of body measures were carried out by using Lidthin stick and a measuring tape. By using Lidthin stick the following measures were determined: wither height (WH), height at hip (HH), body length (BL), chest depth (CD), chest width (ChW), croup width (CW). A measuring tape was used to measure: head length (HL), width of forehead (WF), chest circumference (CC), croup length (CL), fore-cannon circumference (FCC), fore cannon length (FCL), rear cannon length (RCL) and body weight (BW).
Based on the average values of the body measures (Chest circumference and Body length) the body weight of studied population of domestic hilly horse was calculated by using the formula described by *Pejic (1996)*:
BW, kg = CC2 x BL / 11877
In order to describe physical development in more detail, especially body proportions (the relative ratios of certain parts of the body), the indices of body conformation were calculated from the mean values of body measurements.
All collected data were systematized, numerical measurements statistically analysed by using the program Statistica 10.

Results and discussion

Current situation of the horse population

Until the sixties of the last century the horse population had been increasing, due to the fact that agriculture, especially livestock farming, was one of the main economic activities, where horses with oxen were the main work animals. However, restructuring of the economy towards the development of industry and especially wide mechanization of the agriculture have caused sharp decrease in the number of horses. The largest horse population in Montenegro was in the sixties of the last century, reached about 32,000 animals, and since then has been steadily decreasing, so that today's horse population is about 5,000 heads, or about 15.6% of the population in the sixties (table 1). The decreasing trend in the horse population has slowed, but not stopped. In the last 5 to 7 years number of the horses is rather stable.

Table 1: Trend in the size of horse population in Montenegro

Year	1960	1970	1980	1990	1995	2000	2005	2008	2010	2013
Number of animals	32000	29430	25284	20009	16209	12474	7119	5124	4828	4858
Index: 1960 = 100%	100	92	79	62.5	50.6	39	22.2	16	15.5	15.6

According to Census of Agriculture from 2010, the majority of the horse population is on the north, very mountainous region of Montenegro (Andrijevica, Berane, Bijelo Polje, Pljevlja, Plav, Savnik, Zabljak and Pluzine) where 79% of the total population is reared, while in the central region (Podgorica, Danilovgrad, Cetinje and Niksic) is about 19.5%, and the smallest number of horses bred in the south - the coastal region, only 1.5%. In the northern region of Montenegro horses are mainly kept for occasional use for the carrying of loads or just for hobby riding. On the other hand, in the central region of Montenegro, horses are traditionally used 4-5 months per year for transport (carrying out) of firewood from inaccessible terrains, and the rest of the year are practically not used. However, today is more and more frequent use of horses for the purpose of recreational riding in the tourist or therapeutic purposes.

In terms of breed structure of the horse population in Montenegro, domestic hilly horse is dominated; it is estimated about 70% of the total population, while the rest are crosses of domestic hilly horse with modern warm-blooded or cold-blooded breeds.

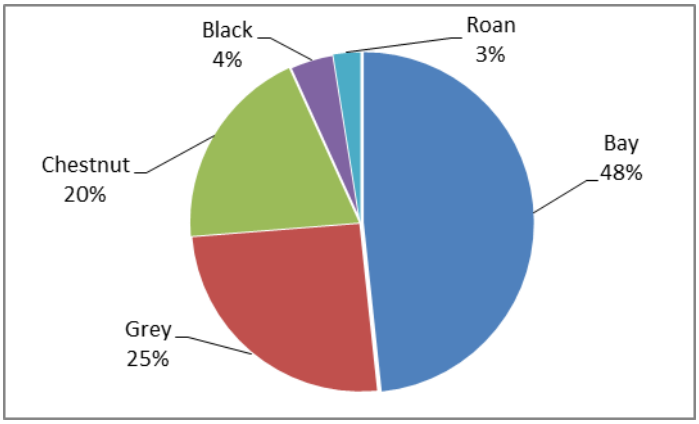
Today's population of domestic hilly horse in Montenegro is very small, especially the pure blood heads, and it certainly is at risk of complete extinction in the long run, especially if we take into account the present negative trends.

Exterior of Domestic hilly horse

Domestic hilly horse from Montenegro, as a part of the population of Balkan hilly horse, in terms of exterior is quite similar to the most studied and described Bosnian hilly horse. The formation of the present type of hilly horse in Montenegro was affected by sporadic and unplanned crossbreeding with other breeds, and the most influence had come from Arabian horse, which came in this region even with the Ottoman Empire. As a result, today's Montenegrin hilly horse has become a slightly bigger and nobler than the horse from earlier periods, which especially refers to the part of the population of the northern region.

Although the horse coat colour is not a breed characteristic, assessment of the participation of certain colours and characteristic signs are important exterior traits in horse breeding in general. The hair colour is, as well as the marks often allow quick identification of individuals, and thus a tool in easier determination of the animal identity.

In the studied population of Domestic hilly horse in Montenegro the most often colour is bay (48.03%) with various shades (light, dark or red bay), than is grey in 25.42%, chestnut was identified in 23 animals or 19.5%, while the black and roan were the colours with the lowest participation (4.2% and 2.5%, respectively).



Graph 1: The presence of the colour of coat in the population of Domestic hilly horse

The presence or absence of specific unpigmented or white pigmented areas on the body, so called marks, commonly found on the head and legs, is an important characteristic of the horse exterior.

Table 2: White marks on the heads of the horse

Marks	Faint	Star	Stripe	Blaze	Without marking
Number of animals	26	12	16	6	58
%	22,04	10,17	13,56	5,08	49,15

The most common white mark on the head of the studied population of domestic hilly horse was faint (small irregular shape) that is identified in 26 animals (22%), then stripe follow (13,56%), while star was determined in 12 animals (10,17%) and blaze in only 5% of the horses, as it presented in the table 2. Without any mark on the head there were nearly half of the horses (49.15%).
The marks on the legs were identified in 32 animals or 27% of studied horses (table 3). The most common mark was pastern (7 animals) and combination of more marks (11 animals).

Table 3: The marks on the horse legs

Marks	Heel	Coronet	Pastern	Stocking	Marks combination	Without marks
Number of animals	1	7	9	4	11	86
%	0,85	5,93	7,63	3,39	10,17	72,88

Body measures of Montenegrin domestic hilly horse

Measures on the head (HL and FW) are very important for determination of size and shape of horse' head. The average HL of the studied population of Domestic hilly horse in Montenegro was 59.4 cm, while the average WF was 20.3 cm (Table

4). Wide range of variation in HL (51 – 65 cm) and its average value higher than the results obtained many years ago on Bosnian mountain horse by: Malecki 1923, Bartolović 1961, Ogrizek and Hrasnica 1952, indicate that probably the Arab or other horse breeds (thoroughbred and coldblood) have influenced Montenegrin population of hilly horse in the meantime. The determined values of WF are compatible with the results of the previous research of above mentioned authors on different populations of hilly horses in the Balkan Peninsula.

Table 4: Body measures and body weight of Domestic hilly horse (n = 118)

Body measures	$\overline{X}$	SD	CV %	Min.	Max.
Head length (HL)	59.44	2.33	4.37	51.00	65.00
Forehead width (FW)	20.34	1.16	5.70	18.00	23.00
Withers height (WH)	134.16	3.32	2.47	123.00	140.70
Hip height (HH)	133.51	3.37	2.52	120.00	140.10
Body length (BL)	139.30	4.85	3.48	127.10	152.50
Chest circumference (CC)	163.53	7.79	4.76	145.00	184.00
Chest depth (CD)	63.50	2.75	4.33	55.30	70.00
Chest width (ChW)	35.35	3.13	8.85	27.80	45.30
Croup width (CW)	46.99	2.62	5.57	41.50	55.00
Croup length (CL)	49.20	2.90	5.89	38.50	57.00
Fore cannon circumference (FCC)	18.68	0.95	5.08	16.50	22.00
Fore cannon length (FCL)	15.13	0.94	6.21	13.00	18.50
Rear cannon length (RCL)	20.19	1.20	5.94	18.00	22.50
Body weight (BW)	314.87	36.61	11.63	232.08	416.18

Withers height, hip height and body length are the measures that determine the body format. The average WH and HH of the studied population of domestic hilly horse were 134.16 cm and 133.5 cm, respectively, what indicates the existence of certain degree of the uphill. BL (139.3 cm) was approximately 5-6 cm longer than WH and HH, what indicates a slightly rectangular body format of the studied horse population. Comparing these results to the results of the research on the populations of mountain horses in Bosnia and Herzegovina and in Mediterranean part of Croatian (*Belić 1940, Habe and Telalbašić 1988, Bartolović 1961, Malecki 1923, Ivanković et al. 2012. and Čačić 2005*) it can be concluded that BL of the Montenegrin population of horses is greater even for 5 to 10 cm.

All the measures determined on the chest (CC, CD, ChW) were slightly higher than the measures determined until now on the hilly horse population from neighbouring countries.

The average CL was only 2 cm larger than the CW, which coincides with the statement of *Mitrović and Grubić (2003)* that the croup of the Bosnian mountain horse well developed and relatively short, square forms, but in terms of providing better care and nutrition they are rounded and slightly downcast.

Fore cannon circumference (FCC) along the FCL and RCL significantly affect the quality and safety steps of domestic hilly horse, work ability and indicate its

constitution type. The average FCC of 18.7 cm indicates rougher constitution of studied horse population. These results are close to results cited by *Telalbašić et al. (1973)* and *Habe and Telalbašić (1988)* for Bosnian mountain horse in stable breeding (17.42 and 18 cm), as well as to Konik and Hucul, autochthonous horse breeds reared in Poland (18 and 19.10 cm), *Komosa and Purzyc (2013).*

Calculated value of body weight was 314 kg in average, but with very broad range of variation (from 232 to 416 kg). It could be caused due to different rearing conditions (nutrition and use for work), as well as due to crossing with other breeds.

The values of body index, as well as indices of compactness and boniness indicate that this studied horse population has quite solid and compact body conformation (Table 5). Compared to other breeds the index of compaction and boniness of domestic hilly horse is higher than for example Posavina horse (*Paprika and Ivankovic, 2005; Simčić et al. 2003*), as well as higher than the index of compactness of domestic horse of Croatian Mediterranean (112%), *Ivankovic et al. (2012).*

Table 5. The values of indices of body conformation

Indices	Model of calculation	Calculated value of indices
Index of body	BL / CC x 100	85.18
Index of body format	BL / WH x 100	103.83
Chest index	CD / WH x 100	47.33
Index of work ability in gallop	CC $(m)^2$ x 56 / HH (m)	112.17
Index of work ability walking	CC $(m)^2$ x 95 / HH (m)	190.23
Index of compaction	CC / WH x 100	121.89
Index of boniness	FCC / CC x 100	11.42
Index of body weight	BW / WH x 100	234.69

Based on the value of chest index, Montenegrin domestic hilly horse belongs to a group of warm-blooded horse, for which the value of chest index, according to Simčić et al. (2012), should range from 46.50% to 48.50%. The values of work index representing optimum of load in work (during gallop and walk). It was calculated that studied horse population can be loaded at a trot and gallop by about 112 kg, and when walking even by 190 kg.

Conclusion

The results of this study pointed out the specifics of population of domestic hilly horse in Montenegro. Description exterior and body measures can be the basis of further search and the eventual consolidation of the population. Although the studied population showed some similarity with the Bosnian mountain horse, consider that the Montenegrin domestic hilly horse certainly different from Bosnia's mountain horse.

These results should be a starting point for further characterization at the genetic level, in order to complete the introduction of the population. It is necessary to mention that this population threatened by further decreasing of population size and her biological survival, and it is necessary to urgently take measures to protect.

Morfometrijska karakterizacija domaćeg brdskog konja u Crnoj gori

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Rezime

Gajenje i korišćenje konja je odavnina imalo veliki značaj za poljoprivredu i ruralno stanovništvo u Crnoj Gori. Konji su bili najvažnije radne životinje za različite namene, stalno prisutna u svakodnevnom životu crnogorskog seljaka i važan deo kulturne baštine ruralnih područja u Crnoj Gori.
Cilj ovog rada je da se prikaže postojeće stanje konjarstva, sistem gajenja i način korišćenja, te osnovne morfometrijske karakteristike populacije domaćeg brdskog konja u Crnoj Gori.
Ukupna populacije konja je u stalnom opadanju, posebno nakon 60-ih godina prošlog stoleća, kad je od 32.000 grla u 1960. broj opao na 4850 grla 2013. Na osnovu rezultata istraživanja najčešća boja dlake proučavane populacije je doratasta (48.03%), beli znakovi na glavi su prisutni kod polovine populacije a na nogama kod oko 30% populacije, a najčešći znak na glavi je gruša (22.23%) a na nogama putast i kombinacija više belega. Prema telesnim merama crnogorski domaći brdski konj spada u grupu malih konja, s prosečnom visinom grebena od 134,2 cm, dužinom tijela 139,3 cm, obim grudi 163,5 cm, obim cevanice 18,67 cm, a težina tela je 314,8 kg. Vrednosti indeksa konformacije tela su pokazali da ispitivana populacija konja ima prilično jaku i kompaktnu građu (trup) veoma dobre radne sposobnosti.

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HORMONAL REGULATION OF GROWTH OF DOWNS AND FEATHERS OF DOMESTIC YOUNG GEESE UP TO THE AGE OF 30 WEEKS AND THEIR QUALITY

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Abstract: Downs and feathers of geese are a valuable renewable natural product. They are utilizable as natural insulating and filling material for bedspreads as well as for high-quality winter-clothing. With regard to harvesting of feathers and downs from living geese more knowledge is necessary on cycling processes of the partial moult and on their endocrine control. Therefore, the aim of the present study was to assess the secretion of moult-associated hormones thyroxine (T_4), progesterone (P), estradiol-17β (E2) and testosterone (T) as well as their potential role as a trigger of partial moult with special regard on feather growth. The study was performed with each six male and female goslings. Blood samples were collected weekly during three different time periods of moult: first partial moult (7^{th} to 10^{th} week of life, WL), second (15^{th} to 17^{th} WL) and third (23^{rd} to 25^{th} WL). Plasma hormone concentrations were analyzed by enzyme and radioimmunoassay, respectively. A strong correlation between feather growth and hormone concentration could be only derived for T4, but not for P, E2 and T. During periods of maximum T_4 values, the growth of feathers and downs has been most widely finished and is directly followed by the subsequent period of new feather growth. In conclusion, this study was helpful to obtain more details about endocrine situation during the partial moult in both sexes of growing geese.

Key words: downs and feathers growth, partial-moulting, secretion of hormones, growing geese

Introduction

Downs and feathers are a renewable natural product based on special high quality characteristics and have been used for more than years. *Menesi et al. (1964)* cit. by *Kozak et al. (2010)* reported, that the downs and feathers of geese other waterfowl species are superior in quality. Especially valuable are the feathers and

down of well-fedgeese, which are obtained during the moult *(Adam, 2001).* Goose downs and feathers have a greater capacity to absorb moisture than synthetic and textile fillers *(Metz, 2002).* With special regard to the fill power, downs of the moulting gathering are superior in quality compared to the slaughter plucking.

The removal of the feathers and downs occurs mainly after the slaughter of waterfowl and a small part (approximately 1 to 2%) by live-gathering of geese during moulting (*Kozák*, 1991 and 2010).

A periodical partial moulting proceed in growing geese which concerns only the small feathers (covering feathers and downs), but not the large feathers (*Schleusener, 1952; Kozák, 1991 and Kozák et al, 1992*). At the beginning of partial moult, growing geese lose their downs and feathers of the small plumage, which can be obtained and can give an additional income for small-scale farmers. Unfortunately, the gathering of downs and feathers of live geese comes sometimes into disrepute in the media, because of unscientific arguments and questionable videos. So it has been suggested that the partial-moulting is not simultaneously occurring in all animals and that only a short period for the moult-plucking is available.

Results of the hormonal control of moulting are available in the literature *(Etches, 1996)* by hens. Especially the thyroid-hormone thyroxine, is given a leading role. However, no studies exist on the regulation of partial moulting in growing geese. Therefore, it should be examined on young geese, consists a relationship between the concentration of various hormones in the blood plasma, the downs and feathers growth and the course of partial moulting in growing geese.

For this reason, the following questions were examined:
- 1. At what age of the young goose downs and feathers reach maturity (quills free from blood and tissue residues) and detach from the skin?
- 2. Is there a correlation between the secretion of selected hormones and the degree of feather maturity as well as the course of the partial moulting?

Material and Methods

Each six male and female goslings of a heavy hybrid line were included in the present study. From the fourth to the 30thweek of age, the body weights were recorded weekly and a representative feather and downs sample were removed from the plumage of the body regions breast, abdomen, flank, dorsal-site and from neck. Blood samples were taken for determination of hormone concentrations of the sexual hormones estradiol-17β (E2), testosterone (T), progesterone (P) and the thyroid hormone thyroxine (T_4) in the blood plasma. Blood sampling was performed weekly during three different ages (first through third partial-moult, see

table 1).The plasma hormone concentrations were analyzed by enzyme and radioimmunoassay, respectively. The bio-statistical analysis *(STATSOFT INC., VOL. 5, 1997*) of the hormone thyroxine was carried out by analysis of variance *(ANOVA)* and the paired t-test for comparison between different periods of the partial moult.

Table 1: Blood samples extraction for hormone analysis

Partial moult (time periods)	Week of life
I	7→8→9→10
II	15→16→17
III	23→24→25

Results

Growth and maturity of the downs and feathers

After hatching the goslings wear their Neoptilen dress, which is formed in the development of the egg. The growth of the downs starts at the 2nd weeks of life (WL), the covering feathers during the third week of life. After an intensive growth phase of the downs and feathers from the 4th WL an abrupt growth arrest followed in the 8th WL on the breast, abdomen, back and neck and the 9th WL on the flanks.

From the 11th WL (2nd plumage generation), the 19th WL (3rd plumage generation) and from the 29th WL (4th plumage generation), an intense length growth of downs and feathers started again, followed by a stop growing of the feathers in the 16th WL on back and neck and in the 17th WL on the breast, abdomen and the flanks as well as in the 26th WL on the breast and abdomen and in the 27th WL at the flanks, back and neck. The growing stop of the feather follows the initiation of growth of the new feathers. These grow from the same feather plant and push the old mature feathers out. This can be described as time of feathers mature and commencement of moulting. The growth of downs is completed about one week before the feathers. The feathers and downs of the first plumage generation have their intense longitudinal growth phases between the 4th WL and the 7th WL, of the 2nd plumage generation between the 11th WL and 16th WL and at the 3rd plumage generation between the 19th and the 26th WL.

Hormonal regulation of the partial-moulting

The analyses of sexual hormones estradiol-17β, testosterone and progesterone show, that they have no influence on the moulting rhythm of the

growing young geese (Figure 1 to 3). Between several time-frames the concentration of sexual hormones from both sexes rises significantly. This increase can be close rather on the development of the reproductive organs as to triggering a partial moulting. The concentration of sexual hormones increases significantly between the individual sections of investigation in both sexes. A strong correlation between feather growth and hormone concentration could be only derived for thyroxine (T_4) (Figure 4). During first partial moult, T_4 values significantly increased in both sexes (ganders: 13.1 ± 1.0 vs. 22.0 ± 2.2 nmol/l, p=0.008; geese: 12.6 ± 1.9 vs. 20.3 ± 1.2 nmol/l, p=0.001) from 7th to 8th WL. Maximum plasma T_4 levels were monitored in male goslings only during 8th WL, whereas those of the females remained elevated until 9th WL and declined not until 10th week. During 2nd partial moult, plasma T_4 concentrations slightly increased in the male goslings (15.2 ± 3.3 vs. 18.3 ± 3.3 nmol/l) from 15th to 16th WL, whereas those of the females amounted markedly higher than males as well as compared to the own values during 1st and 3rd partial moult. From 23rd to 24th WL (3rd partial moult), plasma T_4 levels elevated slightly in the ganders (12.8 ± 1.3 vs. 17.5 ± 0.1 nmol/l), but significantly in the geese (13.3 ± 0.9 vs. 25.6 ± 3.2 nmol/l, p=0.006).

Discussion and Conclusions

The results of this study demonstrate that a marked increase of plasma T_4 values is the most important factor which induce the start of each partial moult in growing goslings. During periods of maximum T_4 values, the growth of feathers and downs most widely has been finished but directly followed by the subsequent period of new feather growth. Based on the data of this study, no relation was found between feather growth and the plasma concentrations of the sexual hormones P4, E2 and T. Nevertheless, it can be concluded that partial moult in growing goslings is triggered independently of E2 and T, whereas the role of P4 related to initiation of partial moult is not clear so far.

A recognizable effect of live weight development on feather development and hormone secretion at the time of moulting was also not found. The growth of the feathers and downs is terminated in the 8th /9th week of life, 16th/17th week of life and 26th/27th week of life. Some past studies indicate periods of 6 to 7 weeks between two partial moult (*Kozak, 1991*). In the present study, the periods between the first and second partial moult as well as the second and third partial moult amounted 8 and 10 weeks, respectively. With the cessation of longitudinal growth, the downs and feathers reach the state of maturity, there are no more blood residue in the spool contain and the marrow is dried (glassy-white color of the coil). Two weeks after the maximum thyroxine concentrations, the feathers and downs of the new plumage generation appear during 10th, 18th and 28th WL followed by an

intensive growth. The new feather and down generation begins with the growth and pushes the old feathers respectively down out of the papillae, so that the supply of blood and innervation (supply an organs with nerve tissue) to be interrupted. This can be referred as the time of feather maturity and the beginning of the moult. About one to two weeks later, the downs and feathers are so loose, that they can be harvested by spread finger like a comb without stressing the animals. The entry of the partial-moulting cannot be forecasted exactly ahead. It is only the determination of the feathers maturity by gathering a sample of about 1% of the animals in a herd of same age, allowing the start of harvesting downs and feathers. Gathering of matured downs and feathers from living geese can improve the income of geese farmers. In summary, this study was helpful to obtain more details about endocrine situation during the partial moult in both sexes of growing goslings.

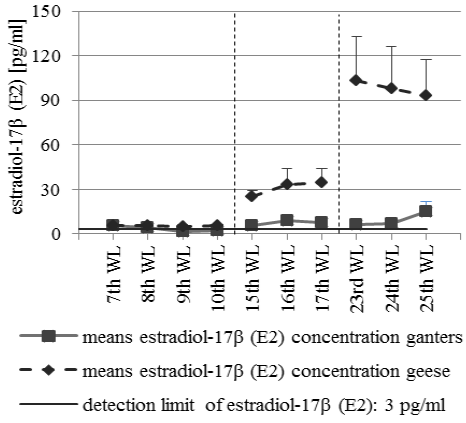

Fig. 1: Development of hormone estradiol-17β by testosterone gander and geese (mean + SEM)

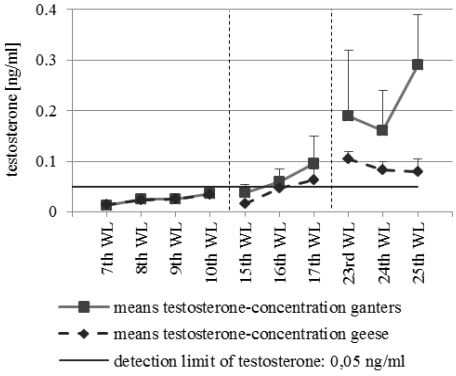

Fig:2: Development of hormone by gander and geese (mean + SEM)

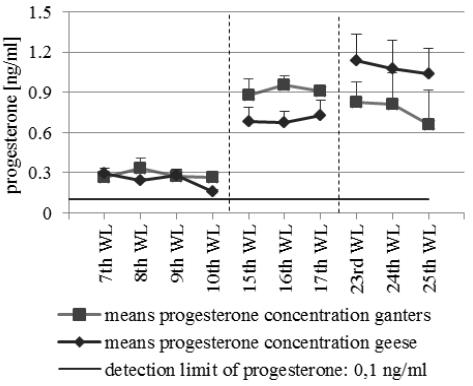

Fig. 3: Development of hormone progesterone by gander and geese (mean + SEM)

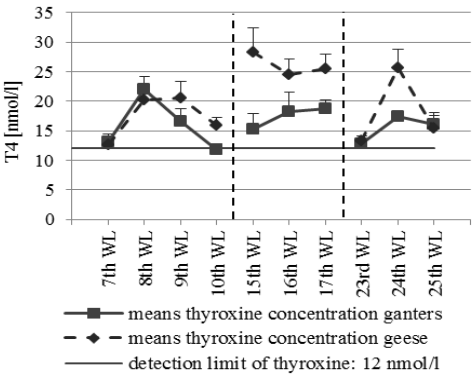

Fig. 4: Development of hormone thyroxine by gander and geese (mean + SEM)

Hormonska regulacija rasta paperja i perja domaćih mladih gusaka do uzrasta od 30 nedelja i njihov kvalitet

M. Waehner, H. Pingel, R. Mueller

Rezime

Paperje i perje od gusaka su dragoceni obnovljivi prirodni proizvod. Oni su iskoristivi kao prirodna izolacija i materijal za punjenje prekrivača, kao i za visoko-kvalitetne zimske-odeće. Što se tiče ubiranja perja i paperja iz žive guske neophodno je više znanja u vezi sa cikličnim procesom delimičnog linjanja i njihove endokrine kontrole. Dakle, cilj ovog rada bio da proceni sekreciju hormona tiroksina (T_4), progesterona (P), estradiol-17p (E2) i testosterona (T) kao i njihove potencijalne uloge kao okidača parcijalnog mitarenja sa posebnim osvrtom na rast perja. Studija je izvedena sa po šest muških i ženskih ptica. Uzorci krvi su sakupljeni nedeljno tokom tri različita vremenska perioda mitarenja: prvo delimično mitarenje (7. do 10. nedelje života, WL), drugo (15. do 17. WL) i treće (23. do 25. VL). Koncentracije hormona u plazmi su analizirane pomoću enzima i radioimunoeseja, respektivno. Jaka korelacija između rasta perja i koncentracije hormona može biti izvedena samo za T_4, ali ne i za P, E2 i T. tokom perioda maksimalne vrednosti T_4, rast perja i paperja je bio u najvećoj meri završen i

direktno mu sledi naknadni period novog rasta perja. U zaključku, ova studija je korisna u smislu dobijanja više detalja o endokrinoj situacije tokom delimičnog mitarenja u oba pola gusaka u porastu.

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EFFECT OF PROLONGED STORAGE OF EGGS OF PEKING DUCKS WITH PERIODICAL WARMING ON INTERNAL EGG QUALITY AND HATCHABILITY

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Invited paper

Abstract: Hatchability decreases as the time of holding of incubation eggs is prolonged. Periodical warming up to 37.4° C for 3 hours on the 3rd day and for 2 hours on the 8th, 11th, 14th, 19th, 21th, 23th 25th and 27th days of storage proved to be a favorable treatment for maintaining hatchability on a higher level compared to storage without warming procedure.

Further investigations should consider the viability of ducklings hatched from eggs stored up until four weeks.

Key words: storage, periodic warming, duck eggs, hatchability, embryonic mortality.

Introduction

Hatchability depends on embryo development between fertilization and hatching. The most important factors for storage of hatching eggs are temperature, humidity, fresh air, egg position and turning. To achieve maximum hatchability it is recommended that eggs stored not longer than 7 days (*Berdougi et al., 2013, Brake et al., 1997*). The negative effects on embryo development caused by storage conditions and duration could be mitigated by pre-warming the eggs during storage.

Only few studies have investigated the effects of storage duration with periodic warming *Bogenfürst (1987, 1995), Pingel and Vagt (1989).*

In pedigree breeding it would be advantageous to prolong the storage time to have a higher number of ducklings with the same age from one breeding duck. Therefore, it is sometimes necessary to store eggs up to 3 or 4 weeks. Longer storage of hatching eggs requires specific treatment like temporary warming during storage.

Bogenfürst (1987) has stored eggs of geese for 10, 17 and 24 days. At the first day of storage and every 5 days the eggs of experiment were warmed up to 37.8° C during 5 hours. Up to the storage of 17 days hatchability was 80.5 % compared to 64.5 % of the untreated control. *Pingel and Vagt (1989*) found that periodical warming of hatching eggs revealed this treatment to be best suited for maintaining hatchability. Warming up to 37.5° C for 2 hours on several days of storage proved to be a favorable method to reach a hatching rate of more than 85 %. Compared to the untreated control the early embryonic mortality amounted to 5.0 % against 15.9 % and the percentage of death in shell was 6.6 % against 16.2 %.

The objective of the present study was to investigate the responses of hatching eggs of Peking ducks to different duration of storage with and without periodic warming.

Material and methods

Hatching eggs for different duration of storage were produced from heavy Peking ducks of the Saifeiya Group, China. The experiments were conducted to find out effects of storage up to 4 weeks with periodical warming on hatchability of duck eggs. For one group of eggs it was applied a periodical warming during storage up to 28 days. The second group was used as control without warming treatment. A total of more than 10 000 eggs from Peking ducks were divided in four groups with storage duration of 1-7, 8-14, 15-21 and 22-28 days.
Warming up to 37.8 ° C has been applied for 3 hours on the 3rd day and for 2 hours on the 8th, 11th, 14th, 17th, 19th, 21th, 23th, 25th and 27th days of storage. The eggs are stored in trays the blunt end uppermost and turned every day. The storage room had a temperature of 12 ° C and a rel. humidity of 80-85 %. The incubation was carried on under standard conditions.

Fertility was evaluated by candling the eggs, carried out at the seventh day of incubation. Hatchability is the percentage of fertilized eggs with development until the hatching of live viable ducklings. The not hatched eggs were assigned to early embryonic mortality, dead in shell and: delayed hatch of not viable ducklings.

Statistical analysis based on Chi-square-test (*Rasch, 1987*).

Results and discussion.

The results of fertility and hatchability of experiment 1 are demonstrated in table 1. The decrease of fertility rate in the group with periodical warming was not significant. The significant decrease of fertility rate in the group without warming can be the result of very early embryonic mortality.

Table 1. Effect of long-term storage with and without periodic warming on hatchability of Peking ducks, exp. 1, 2012.

With warming	1-7 days	8-14 days	15-21 days	22-28 days	Chi-square
Set eggs	715	642	620	614	
Fertile eggs	691	560	548	533	
Fertility, %	90.3	87.2	88.4	86.8	6.675 -
Hatched ducklings	616	487	443	379	
% hatchability	89.1	87.0	80.8	71.1	105.9 ++
Without warming					
Set eggs	800	829	643	647	
Fertile eggs	721	760	570	544	
Fertility, %	90.1	91.7	88.6	84.1	23.5 ++
Hatched ducklings	618	657	399	349	
% hatchability	85.7	86.4	70.0	64.2	138.9 ++

- not significant, + significant (alpha > 0.05)++ significant (alpha > 0.01)

The reduction in hatchability is highly significant in both groups but it was more pronounced in the group without warming. In both groups longer duration of storage has a significant effect on increasing of early embryonic mortality, dead in shell and delayed hatching of not viable ducklings (Table 2), but delayed hatching was more pronounced in the group without warming.

Table 2. Differentiation of not hatched eggs in experiment 1

	Warming	1-7 days	8-14 days	15-21 days	22-28 days	Chi-square
Early embr.	Yes	2.03 14	3.39 19	7.85 43	8.82 47	48.8 ++
mort., %	No	4.85 35	4.08 31	9.65 55	11.21 61	35.9 ++
Dead in	Yes	4.49 31	5.71 32	4.93 27	12.0 64	45.8 ++
Shell, %	No	5.55 40	5.65 43	11.93 68	9.01 49	24.7 ++
Delayed	Yes	4.34 30	3.93 22	6.39 35	8.07 43	14.5 ++
Hatch, %	No	3.88 28	3.82 29	8.42 48	15.63 85	100.3 ++

++ significant (alpha > 0.01)

Table 3 shows the results of experiment 2 in 2013 with a smaller control without warming.

The fertility was not influenced by storage in the group without warming. In the group with warming the fertility was significantly reduced in the third and fourth week of storage.

Table 3. Effect of long-term storage with and without periodic warming on hatchability of Peking ducks, exp. 2, 2013.

With warming	1-7 days	8-14 days	15-21 days	22-28 days	Chi-square
Set eggs	657	775	1071	1253	
Fertile eggs	490	555	688	793	
Fertility, %	74.6	71.6	64.2	63.3	35.9 ++
Hatched ducklings	390	433	530	551	
% hatchability	79.6	78.0	77.0	69.5	21.29 ++
Without warming					
Set eggs	168	170	220	273	
Fertile eggs	126	122	165	205	
Fertility, %	75.0	71.8	75.0	75.1	< 1 -
Hatched ducklings	92	86	81	101	
% hatchability	73.0	70.5	49.1	49.3	33.5 ++

++ significant (alpha 0.01)

Longer storage had a significantly reducing effect on hatchability. In the group with periodic warming it was due to increasing early embryonic mortality, but in the group without warming it depends mainly on the increasing percentage of delayed hatching, as can be seen in table 4.

Table 4. Differentiation of not hatched eggs in experiment 1

	Warming	1-7 days	8-14 days	15-21 days	22-28 days	Chi-square
Early embr. mort., %	Yes	3.06	4.56	5,81	12.61	55.8 ++
	No	3.70	3.92	7.88	9.76	6.24 -
Dead in Shell, %	Yes	6.73	5.95	6.69	6.94	< 1 -
	No	7.34	7.84	6.67	13.66	5.75 -
Delayed hatch, %	Yes	12.44	12.79	10.90	11.35	1,35 -
	No	16.67	17.03	34.54	27.32	16.33 ++

++ significant (alpha 0.01)

Conclusions

In both experiments the negative effect of storage duration on hatchability was more pronounced in the group without periodic warming. From the results of periodic warming it can be recommended to prolong the duration of storage up to three weeks. Especially, early embryonic mortality and delayed hatching have caused the decrease of hatchability. In further investigations it has to be investigated the effect of long storage of eggs on the quality of the hatched ducklings and their growth potential.

Uticaj produženog skladištenja jaja pekinške patke sa periodičnim zagrevanjem na unutrašnji kvalitet jaja i procenat izleganja

M. Waehner, H. Pingel, S. Haidong

Rezime

Procenat izleganja se smanjuje kako se vreme održavanja inkubacije jaja produžava. Periodično zagrevanje na 37,4°C u trajanju od 3 sata 3. dan i u trajanju od 2 sata 8., 11., 14., 19., 21., 23., 25. i 27. dana skladištenja se pokazalo kao povoljniji tretman za održavanje procenta izleganja na višem nivou u odnosu na skladištenje bez postupka zagrevanja.

Dalja istraživanja treba da razmotre održivost pačića izleženih iz jaja uskladištenih do četiri nedelje.

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IMPORTANCE OF FARM MANAGEMENT IN REDUCING BROILERS SKIN LESIONS

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Invited paper

Abstract: The incidence of contact lesions in broiler production is showing an upward trend over the past decades. The frequency and severity of lesions are perceived through the negative effects on productivity, carcass quality and broiler welfare. High variability of the frequency of contact lesion between farms indicates a significant influence of management and applied technological procedures on farms. The most common forms of contact lesions are on the feet, ankles and breast and the areas of direct and prolonged contact with the litter. In this regard, a number of studies have pointed to the significant effect of litter on the health and integrity of the skin. In addition to litter, farm factors that are associated with the incidence of contact lesions are stocking density, environmental conditions, light program, nutritional factors, etc. The paper gives an overview of recent research aimed at reducing the incidence and severity of contact lesions by implementing of certain technological processes with effects on the quality of the litter.

Key words: broiler, contact lesions, farm management

Introduction

Lesions on the skin of broilers are common inflammatory damage to the various layers of the skin. Primarily they are not caused by microorganisms but can be a place of entry of bacteria (*Hester, 1994*). They occur on the feet, ankles and breast of broilers, i.e. in places of prolonged contact of skin and litter. Therefore, they are associated with quality of litter, conditions and farm management (*Shepherd and Fairchild, 2010; de Jong et al., 2012*). According to the report SCAHAW (2000) occurrence of contact dermatitis is an increasing problem. The reasons are in the industrialization of poultry production and increase of the growth intensity of

hybrids, which resulted in a greater burden on farms, less physical activity and poorer state of broiler litter.

The incidence of skin lesions in broilers is considered to be an economic and welfare problem (*Shepherd and Fairchild, 2010; Hashimoto et al., 2013*). Lesions diminish the carcass quality at slaughter line. The lesions on the plantar surface of the foot (footpad - FPD) are the most common forms that directly diminish the quality of chicken legs, a product most in demand in the Asian market, thereby reducing the efficiency of the carcass. More serious forms of foot lesion are the main causes of painful conditions, hampering movement and access to feeders, which leads to negative effects on body weight gain (*Škrbić et al., 2009a; 2011; Hashimoto et al., 2013*).

Data on the frequency of contact lesions on farms are limited. A realistic assessment is difficult due to different, subjective grading systems that are causing great variability of results obtained in individual farms (*Allain et al., 2009*). The methods of visual assessment of the scope and depth of the lesions that are used quantify the lesions with points according to the scale of different range (tab. 1). *Arnould and Butterworth (2010),* state variability of lesion incidence 0-100% depending on the flock. The information cited by other authors on the frequency of FPD on commercial farms range from 30% (*Sanotra et al., 2003*); 38% (*Ekstrand et al., 1997);* 11.2% with the variability of 0 to 71.5% (*Haslam et al., 2007*).

Table 1. Several scoring systems for foot lesions

	Score	Description
Ekstrand et al., 1998	0 1 2	No lesions, only mild hyperkeratosis, no discoloration Mild lesions; superficial lesions, erosions and discoloration Severe lesions; deep lesions and ulcers
Dawkins et al., 2004	0 1 2	No lesions < 5mm lesion on pad > 5mm lesion on pad
Thomas et al. 2004	1 2 3	No burns Mild burns Severe burns
Allain et al., 2009	0-9	Scoring system based on a combination of severity and surface area

Great variability of the frequency of contact lesions between farms reflects the different growing conditions, management techniques and broiler genotypes. In order to prevent contact dermatitis mandatory monitoring in slaughterhouses is essential as well as understanding of the breeding factors in farms that are associated with the incidence of the lesions, to improve the farms management

practices (*Directive 2007/43/EC*). Factors associated with the incidence of contact lesions in broilers are litter quality, stocking density, light program, nutritional factors, breeding system, age, body weight, genetic factors (*de Jong et al., 2012; Bilgili et al., 2006; Eichner et al., 2007; Škrbić et al., 2009a; Kjaer et al., 2006*). Great variability of contact lesions between farms (*Haslam et al., 2007*), indicates the possibility for reduction of the prevalence of lesions by improving the technological aspects of production.

Litter quality

The quality of litter is extremely important for the state and health of the skin of broilers because of the time they spend in contact with her. Litter quality influences the environment conditions in the facility: humidity and air temperature, ammonia level and the amount of dust in the air. The role of litter is in thermal insulation and comfort and convenience that have to be provided for broilers. Due to poor maintenance of its quality this often turns into its opposite, so moist litter in prolonged contact with the skin results in the appearance of lesions. In several studies wet litter was identified as the main cause of lesions (*Shepherd and Fairchild, 2010; Kyvsgaard et al., 2013; de Jong et al., 2014*). The wet litter facilitates the adhesion of faeces on the skin, which in addition to mechanical enables the chemical irritation. Lesions are therefore sometimes referred to as ammonia burns. Quality of litter largely determines the moisture content in it. In this sense, *Mayne et al. (2007), Youssef et al. (2010), Cengiz et al. (2011)* state that only wet litter induces dermatitis. However, in some studies, despite the lack of a statistically significant difference in the moisture content of the litter between the treatments, significant differences in the incidence of lesions were found (*Škrbić et al., 2015*). The reason was in the other, physical characteristics of litter that affected the looseness and softness (fineness), i.e. hardening and caking of litter. In this sense, the choice of material for litter is extremely important. Criteria for selection are its absorptive capacity, the speed of drying, caking, availability and price. The most commonly used materials are straw and wood shavings, but due to the rationalization of production alternative materials are tested such as sand, paper, rice husk, etc. (*Bilgili et al., 2009*; *Grimes et al., 2006*). By comparing of alternative and traditional materials, *Grimes et al. (2006)* have not found significant differences in the incidence of lesions on the feet, while *Bilgili et al. (2009),* based on absorption capacity and speed of drying, preferred the ground door filler or sand. In numerous comparisons of straw and wood shavings, lower frequency of lesions was found in the treatments with wood shavings (*Su et al., 2000; Sirri et al., 2007; Meluzzi et al., 2008a*). In the study by *Škrbić et al. (2015)* significant differences in the occurrence of lesions between the chopped straw and

wood shavings were established already at the age of broilers 21 days, although it is known that the incidence of lesions increases significantly with the age broilers (*Kjaer et al., 2006*) due to worsening conditions in the facility. Larger litter particles have a direct negative effect on the development of footpad lesions (*Cengiz et al., 2011*). In this respect, the recommendation is chopping of straw (*Bjedov et al., 2013*), which may contribute to faster aeration. An absorptive capacity of the litter can be improved by greater thickness of the layer, i.e. using a larger amount of material per unit area. *Meluzzi et al. (2008a)* have found that higher amounts of litter (3-4.5 kg/m^2), with a lower stocking density and shorter photoperiod, contribute to a lower incidence of footpad lesions. On the other hand, the layers of litter thinner than 5 cm may result in a lower incidence of footpad lesions compared to thicker layers because the thinner layer litters provide better aeration through pecking and activity of chickens. In addition, the thin layers of litter can be fully ventilated using ventilation systems (*Ekstrand et al., 1997*).

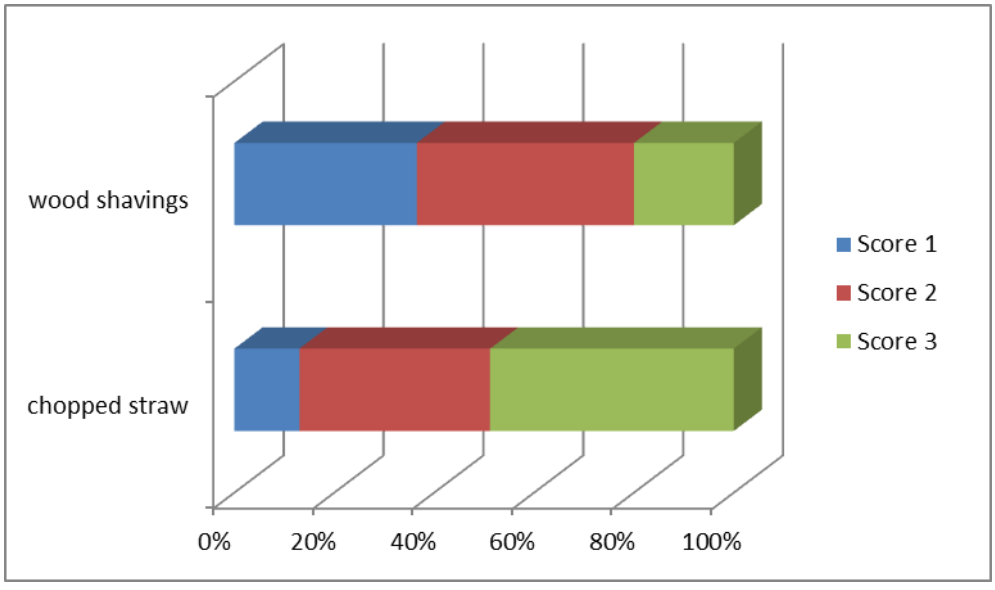

Figure 1. Frequency of foot-pad lesions scored from 1(without lesions) to 3 (severe lesions) in different litter type (*Škrbić et al., 2015)*

Environmental conditions

Basic environmental parameters in terms of litter quality are temperature and humidity. The optimal ventilation level is based on these parameters. With

increasing temperature the absorptive capacity of the air increases. In this sense, the season has a significant impact on the occurrence and severity of lesions. Statistical models in the study of *de Jong et al. (2012)* have suggested a variation of heavy footpad lesions of 13% depending on the season in which day-old chicks were housed. The highest incidence of footpad lesions was recorded in flocks where chickens were housed in the period December-March and the opposite, the lowest incidence was in flocks which have been housed in the period June-August. Early exposure of chickens to wet litter increases the occurrence and severity of lesions (*Cengiz et al., 2011; Taira et al., 2014*). Effects of season were also established by *Haslam et al. (2007), Meluzzi et al. (2008b), Musilova et al. (2013).* The severity of lesions was reduced in hot, dry months and to the contrary, increased in the colder months. The basic problem in regulating the ambient conditions during the winter period is the pursuit of saving heat through restrictive levels of ventilation, which results in an increase in air humidity.

Stocking density

Numerous studies confirm the role of stocking density in achieving the production performance and the preservation of the welfare of broilers (*Škrbić et al., 2009a*). However, in regard to the direct correlation with the incidence of contact lesions, the results are conflicting. Some studies suggest an association between high stocking density and higher frequency of lesions (*Haslam et al., 2007*), while others suggest that the stocking density cannot be considered a significant factor (*Meluzzi et al., 2008b*). The main effect of stocking density on lesions is low in the study of *Kyvsgaard et al. (2013),* but there is a significant interaction with the season in the sense that the high stocking density leads to more serious lesions in the summer and it has a slightly protective effect during winter. By limiting the living space and are to move, high stocking density prolongs chicken contact with litter and prevents the drying. In addition, at high densities the moisture content of the litter increases, increasing the burden of faecal matter and ammonia, which allows microbial activity that increases the temperature of the litter (*Meluzzi and Sirri, 2009*). Also, the high stocking density makes it difficult to properly manage the litter. In this way the relationship between stocking density and the occurrence of contact lesions can be explained. The effect of stocking density is seen through changes in the litter quality that was the best in the lower stocking density, resulting in a complete absence of lesions in the study by *Škrbić et al. (2009b).* Based on the incidence of footpad lesions, it was concluded that the limit stocking density is 16 birds/m^2 and that significant differences were found between the density of 10 birds/m^2 and 20 birds/m^2 (*Škrbić et al., 2010*). However, given that the negative effects of high stocking density may be compensated by improving

environmental conditions, *Dawkins et al. (2004)* find that stocking density has little effect on the occurrence of footpad lesions. In support of this are the current limits of stocking density of broilers in accordance with the conditions for the preservation of the welfare that reduce the effect of stocking density on the development of contact lesions (*Kyvsgaard et al., 2013*).

Lighting program

Improving of broiler welfare conditions includes mandatory introduction of the dark periods during the 24-hour cycle in order to establish a clear scheme of entirely separate periods of light and dark. In this way a normal secretory pattern of melatonin is ensured and metabolic disorders reduced (*Apeldoorn, et al., 1999*) and this also contributes to the health of the legs (*Sanotra et al., 2002*). Various aspects of lighting program (photoperiod, intensity and colour of light) can have different stimulatory effect on physical activity of broilers (*Lewis and Morris, 1998*). The research results of *Schwean Lardner et al. (2012*) show that broilers reduce their physical activity with extending of the photoperiod. The duration of photoperiod did not show a significant effect on the occurrence of footpad lesions in the work of *Sirri et al. (2007)* where lighting programs 23L: 1D and 16L: 8D were compared. In contrast, the inclusion of several short periods of darkness during the photoperiod, i.e. the application of intermittent lighting programs leads to greater physical activity of broilers in the periods of light and reduces the time of contact with the wet litter. It is assumed that in this way the expression of certain forms of behaviour are also influenced, such as pecking and scratching (*Maddocks, et al., 2001),* and thus less compaction of litter, better drying and finally its better quality are provided. Lower incidence of the worst forms of footpad lesions was found in the gradual extension program in relation to the program of consistently reduced photoperiod (Fig. 2), due to the simultaneous effect of the intermittent lighting that is achieved by gradual extending of photoperiod (*Škrbić et al., 2015*).
The low light intensity increases the incidence of lesions, influencing the activity of chickens which decreases with simultaneous prolonging of the contact with the litter (*Blatchford et al., 2009*). Similar results are reported by *Deep et al. (2010)* for the light intensity of 1 lux compared to 10, 20 and 40 lux, while this effect did not occur in the study of *Kristensen et al. (2006)* who compared the light from a different source and the intensity of 5 and 100 lux.

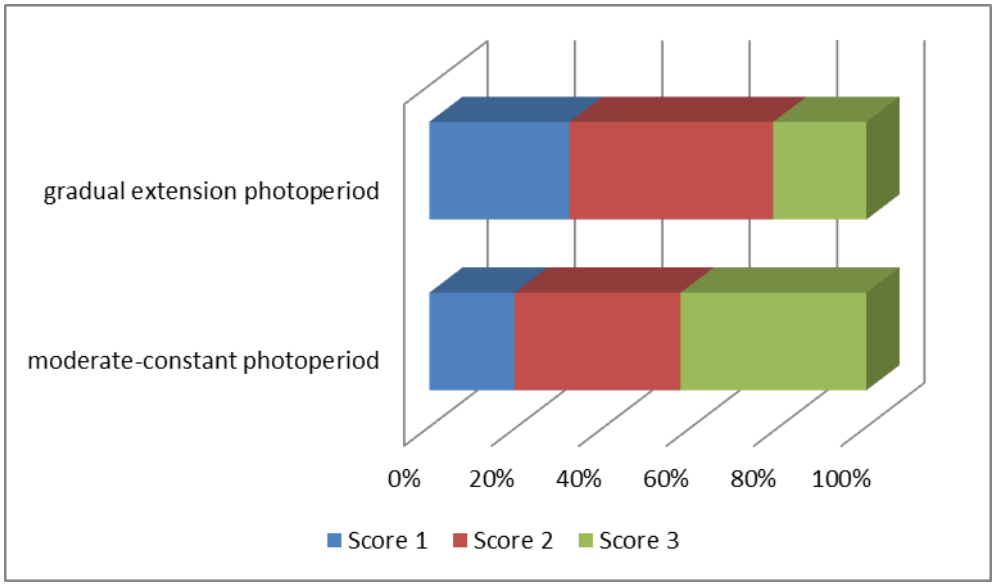

Figure 2. Frequency of footpad lesions scored from 1(without lesions) to 3 (severe lesions) affected by lighting program (*Škrbić et al.*, 2015)

Nutrition

The effect of nutrition on the occurrence of contact lesions involve different sources and levels of protein, diet density, vitamin-mineral supplementation, the use of enzymes, etc. Nutrition exerts its effect through consistency of faeces which largely determines the quality of humidity of litter (*Francesch and Brufau, 2004)*. On the other hand, the deficit of vitamins, biotin and riboflavin, as well as sulphur amino acids, methionine and cystine, acts on the structural components of the skin and facilitates the formation of contact dermatitis (*Mayne et al., 2005*). Biotin deficiency can occur when the soy meal is used as a single high protein raw material in the mixture as it is naturally deficient in biotin. However, supplementation of biotin did not reduce the incidence or severity of the lesions in the work of *Mayne et al. (2007)* in view of the established interaction between the effect of supplementation and litter quality. High levels of Na and K contribute to greater consumption of water and litter moisture. The use of certain raw materials, such as soy, due to high levels of K, if they are not balanced, can lead to increased water consumption and wetter litter (*Cengiz et al., 2012*). The high level of crude protein in the diet leads to high levels of uric acid discharged by kidneys, which stimulates the consumption of water and results in wet faeces. Diets with a high percentage of plant proteins imply increasing of the amount of fermentable

carbohydrates, resulting in an increased faecal viscosity and gluing to the foot pads, which is a problem even in the case of an acceptable moisture content in the litter (*Nagaraj et al., 2007a*). Using commercially available enzymes can result in increased digestibility of such mixtures (*Nagaraj et al., 2007b*). Formulating a diet based on digestible amino acids, and the addition of synthetic, will ensure that the nutrient content meets the needs of the broiler with no negative effect on the quality of litter and incidence of lesions. Low diet density has caused significantly lower incidence of footpad dermatitis in comparison to the high diet density in the study of *Bilgili et al. (2006)*, due to reduced content of soybean meal and hardly digestible fats (*Mukrami et al., 2000*), which reduced the faecal viscosity. The role of Zn in the skin regeneration and health can be improved by the use of organic sources (*Saenmahayak et al., 2010*). In the study by *Škrbić et al. (2012),* the use of mixtures which, based on the raw material composition, are declared as market efficient led to higher humidity of litter and significantly higher incidence of footpad lesions relative to the mixture with a standard raw material composition (fig. 3).

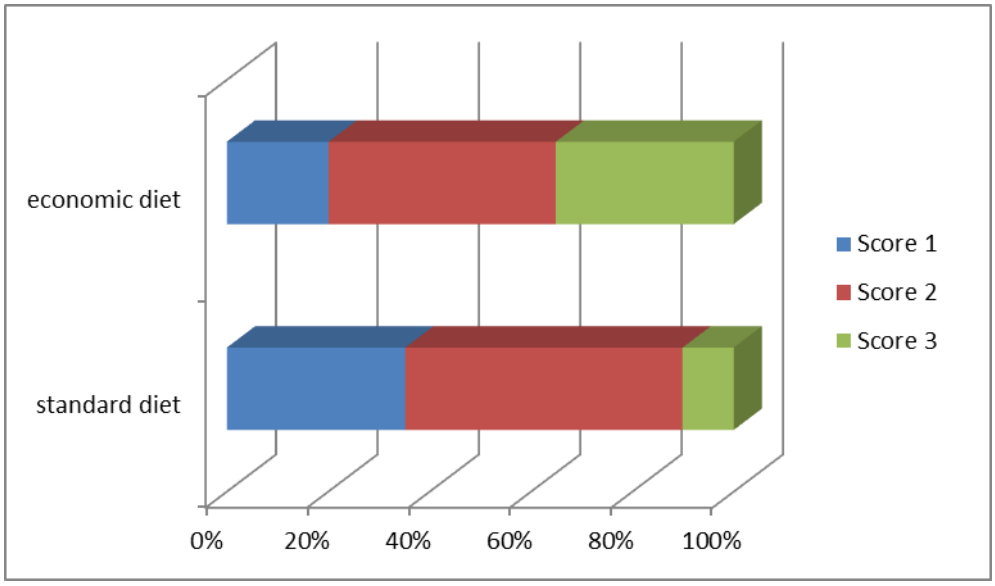

Figure 3. Effect of diet on incidence of foot-pad lesions scored from 1(without lesions) to 3 (severe lesions) (*Škrbić et al., 2012)*

Conclusion

The incidence of lesions in broiler production is seen as a problem with effects on the quality of the whole carcass, food safety and animal welfare, which is causing a growing interest in defining the cause in an effort to solve this problem. The problem of the incidence of lesions in broiler production is showing an upward trend in recent decades. The frequency and severity of certain forms of lesions is usually correlated with the litter quality. Research reports indicate the possibility of reducing the incidence and severity of lesions by applying certain technological procedures with impact on the quality of the litter.

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Značaj menadžmenta farme u redukciji kožnih lezija u brojlera

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Rezime

Incidenca kontaktnih lezija u brojlerskoj proizvodnji je sa tendencijom porasta tokom proteklih decenija. Učestalost i ozbiljnost lezija sagledava se kroz negativne efekte na produktivnost, kvalitet trupa i dobrobit brojlera. Velika varijabilnost učestalosti kontaktnih lezija između farmi ukazuje na značajan uticaj menadžmenta i primenjenih tehnoloških postupaka na farmama. Najzastupljeniji oblici kontaktnih lezija su na stopalima, skočnim zglobovima i grudima, odnosno na mestima direktnog i produženog kontakta sa prostirkom. U tom smislu, brojnim studijama je ukazano na signifikantan uticaj kvaliteta prostirke za zdravlje i integritet kože. Pored prostirke, faktori farme koji se dovode u vezu sa pojavom kontaktnih lezija su gustina naseljenosti, ambijentalni uslovi, svetlosni program, nutritivni faktori. U radu je dat pregled sprovedenih istraživanja sa ciljem redukovanja pojave i

ozbiljnosti kontaktnih lezija primenom određenih tehnoloških postupaka sa efektima na kvalitet prostirke.

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EFFECT OF "WOODEN BREAST" APPEARANCE ON POULTRY MEAT QUALITY, HISTOLOGICAL TRAITS AND LESIONS CHARACTERIZATION

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Invited paper

Abstract: The purposes of the study were to investigate the effects of Wooden Breast (WB) myodegeneration on poultry meat quality and to give a contribution in typing lesions morphology. At a poultry meat cutting facility, 30 normal (N) and 30 affected (WB) breast fillets (*Pectoralis major*) were selected. Weight, cross sectional area (CSA), pH, L*a*b* colour values, water-holding capacity and Warner-Bratzler shear force were determined. Samples were also visually and histologically evaluated. Affected samples were heavier, thicker, paler ($P<0.001$) and characterized by palpatory hardness and lower water holding capacity ($P<0.05$). Macroscopically, abnormalities were primarily localized in the cranial portion of the fillet and defined by the presence of bulges, petechiae, fluid and clear exudate and higher pH. Microscopically, the WB condition was characterized by cells with greater CSA ($P<0.001$) and higher giant fibres prevalence ($P<0.01$). Data suggest a relationship between breast weight and WB condition.

Key Words: Poultry meat quality, Wooden Breast, Myodegeneration

Introduction

An ever-increasing demand of chicken meat has enforced poultry supply chain to improve growth rate and breast meat yield of broilers, but this trend led to a substantial onset of breast muscle defects at the same time. An emergent myopathy has been described and named Wooden Breast *(Sihvo et al., 2014)*.

Distinguishing macroscopical traits of the affected *Pectoralis major* muscle are the remarkable palpatory hardness and the pale colour, as well as the presence of bulges, small hemorrhages and a slimy surface due to a fluid and clear exudate covering the hardened areas. Interestingly, also the observation of White Striping (WS) and extended areas with separated muscle bundles is often concomitant. Histologically, WB condition is a moderate or severe myodegeneration accompanied by abnormalities in cells structure and shape (internalization of nuclei, split cells appearance and loss of polygonality), necrosis, inflammatory cells accumulation and reparative responses (connective tissue proliferation or fibrosis) *(Sihvo et al., 2014)*. Little is still known about WB formation mechanisms and its quality implications; nevertheless, this condition demonstrates similar histological traits as WS, whose aetiology has just been explored even if not completely. The greatest suspicions seem to fall on the fast growth rate and ever-increasing breast meat yield reached by birds, which make muscle to be prone to develope overstretching and capillary density lowering; ischemia resulting in inflammation and reparative fibrotic responses is the consequence *(Kuttappan et al., 2013)*. The aim of the present study is to deepen the knowledge of the impact of WB on chicken breast meat quality and to furnish additional information on the lesion morphology, both at visual and microscopic levels.

Materials and Methods

At a poultry-meat cutting facility, 30 normal (N) and 30 affected (WB) breast fillets were selected 48h *post mortem*, after the visual and tactile inspection from 474 carcasses. WB condition was observed in 252 carcasses, which represented the 53.2% of the examined carcasses.

Affected samples were visually examined for the WB descriptors: colour, consistency, bulges localization and appearance of exudate and hemorrhages. Selected right breasts were weighed; on their cranial and caudal ends, pH was determined using Mettler Toledo FE20 pHmeter and colour values of lightness, redness, yellowness, chroma and hue (L*a*b*, C* and H°, respectively) were measured with RM200QC colorimeter (X-Rite, Co, Neu-Isenburg, Germany). Samples were individually vacuum-packed and kept frozen for 3 months at -20 °C; thereafter, fillets were thawed to determine the thawing losses, again vacuum-packed and cooked in a water bath until core temperature of 74 °C to determine the cooking losses. Shear force was assessed with TA-HDi Texture Analyzer (Stable Macro System, London, UK) on 6 cylinder-shaped cooked meat pieces per sample (Ø 1.25 cm), cut perpendicularly to the fibres direction by a Warner-Bratzler cell (100-kg load cell, 2 mm/s crosshead speed) inserted in the

texturometer. WBSF values of each sample represent an average of the 6 measurements.

Cross Sectional Area (CSA) of the fillet and histological observations were performed on the left half breasts. CSA data were obtained cutting transversely the cranial portions and photographing the views with Canon EOS 500D camera with 18-200 mm/3.5-5.6 lens; images were subsequently processed with digital image software (Carl Zeiss, Model Axiovision 4.6.3.0).

Breast samples (15 N and 15 WB) were taken from the central-external part, fixed in 10% formalin, processed by conventional methods and embedded in paraffin wax and cutting them at a 4-µm thickness for Hematoxylin-Eosin (HE) staining (to estimate average normal fibres and giant fibres number and size) and for Masson's trichrome staining (to measure fibres CSA, collagen and empty spaces percentages).

Computerised image analysis *(Buche, 1990)* was used to determine the fibre mean CSA (of both normal and giant fibres), the collagen and empty space area, and to count the average frequency of giant fibres per unit size of muscle section.

The statistical software SAS (2004) package (version 9.3) was used. Breast quality traits, CSA, weight and histological data were analysed through ONE-WAY ANOVA considering treatment (N *vs*. WB) as independent variable. Descriptors were examined by $\chi 2$ and z tests.

Results and Discussion

At 48h *post mortem*, WB were characterised by significantly higher pH and colour values and these differences had been maintained after the frozen storage. The breast cranial portions suffered more from the WB effect, notable for the higher pH and yellowness index values ($P<0.01$), whereas both the regions were brighter ($P<0.001$), thus confirming the evidence of the importance of this last feature in the WB condition. Chroma (C*) and hue (H°) followed the similar trend of redness index values (Tables 1 and 2).

Table 1. Breast *(Pectoralis major)* pH and L*a*b* colour values at 48 h *p.m.*

	N	WB	Significance	RSD(1)
pH Cranial	5.90	6.03	**	0.19
pH Caudal	5.87	5.92	ns	0.16
L* Cranial	50.9	54.6	***	3.4
L* Caudal	50.5	53.8	***	3.3
a* Cranial	-1.1	-0.3	**	1.4
a* Caudal	-1.2	-0.6	ns	1.4
b* Cranial	12.9	15.9	**	3.1
b* Caudal	12.4	13.3	ns	3.6
C* Cranial	13.0	16.0	**	3.2
C* Caudal	12.6	13.4	ns	3.6
H° Cranial	95.4	90.5	**	5.2
H° Caudal	96.8	94.3	ns	7.3

*: P<0.05; **: P<0.01; ***: P<0.001; (1) Residual Standard Deviation

Petracci et al. (2013a, 2013b) obtained analogous pH values in previous studies on White Striping (WS) condition. The selection for increased yield resulted in hypertrophic breasts with reduced glycogen storage, thus causing higher ultimate pH *(Berri et al., 2004; Le Bihan–Duval et al., 2008)*. Higher L* and b* values may be related to fibrotic responses associated to the myodegeneration.

Table 2. Breast (*Pectoralis major*) pH and L*a*b* colour values after frozen storage

	N	WB	Significance	RSD(1)
pH Cranial	5.91	6.03	***	0.12
pH Caudal	5.86	5.91	ns	0.13
L* Cranial	48.5	52.3	***	3.0
L* Caudal	48.9	51.3	***	2.4
a* Cranial	-0.4	-0.7	**	1.3
a* Caudal	-1.2	-1.2	ns	1.3
b* Cranial	16.7	19.8	**	3.7
b* Caudal	13.9	15.2	ns	3.7
C* Cranial	16.7	19.9	**	3.7
C* Caudal	14.1	15.4	ns	3.6
H° Cranial	92.1	88.8	**	4.6
H° Caudal	96.0	96.0	ns	6.23

**: P<0.01; ***: P<0.001; (1) Residual Standard Deviation

WB fillets were heavier and exhibited greater CSA than N fillets (30.3 *vs*. 25.1 cm^2, respectively; P<0.001) (Table 3). *Brewer et al. (2012)* found a high correlation (r = 0.81) between the two parameters. Our WB fillets also displayed a worse WHC, as the superior cooking losses (P<0.01) contributed to the higher total losses (P<0.05). Muscles have a reduced ability to hold water possibly as a consequence of the myodegeneration, which replace myofibrillar proteins in favour of connective proteins that do not bind water *(Sihvo et al., 2014; Petracci et al., 2013b)*.

Shear force values of the affected cooked breasts did not correspond to the palpatory hardness perceived at the fresh and thawed state. The extensive poor cohesion and the tendency of the fibre bundles to separate mainly at the cranial level could have mitigated the expected hardness *(Petracci et al., 2013b, Petracci and Cavani, 2012)* (Table 3).

Table 3. Breast (*Pectoralis major*) weight, WHC, WBSF and cross-sectional area (CSA)

	N	WB	Significance	RSD(1)
Weight, g	377	505	***	49.7
Thawing losses, %	5.4	5.0	ns	1.7
Cooking losses, %	23.5	26.4	**	3.7
Total losses, %	29.0	31.4	*	4.4
WBSF, N	15.5	16.9	ns	2.8
CSA, cm^2	25.1	30.3	***	4.5

*: P<0.05; **: P<0.01; ***: P<0.001; (1) Residual Standard Deviation

Macroscopically, the descriptors used highlighted that lesions are distributed mainly in the cranial end of the fillets (P<0.001) (Table 4). The presence of bulgies (in 100% of the cases) was one of the selection traits, and it was localized at the cranial level in the 60% of the observations, as well as the hard palpatory consistency and the colour ranging from normal to pale.

The exudate appeared with a prevalence of 43.4% and it was mainly fluid and clear (P<0.01), whereas pinpoint hemorrhages were present in the 30% of the cases and mostly in the upper area. Similarly, also WS displayed higher severity degree toward the cranial side of the breast, where the muscle displayed maximum thickness and, as hypothesized by *Kuttappan et al. (2013)*, also for WB a possible explanation could be found in the overstretching or ischemia resulting in tissue damage and attempt of reparative responses.

Table 4. WB descriptors prevalence

Descriptors				Significance
Localization, %				
Cranial	Caudal	Cra + Cau	Longitudinal	
56.7^{A}	3.3^{B}	16.7^{B}	16.7^{B}	***
Breast colour, %				
Normal	Pale	Pink	Other	
36.7^{A}	56.7^{A}	3.3^{B}	3.3^{B}	***
Breast consistency, %				
Hard	Very hard			
80.0^{A}	20.0^{B}			***
Bulgies localization, %				
Cranial	Caudal	Cra + Cau	Longitudinal	
60.0^{A}	0.0^{C}	23.3^{B}	16.7^{BC}	***
Exudate consistency, %				
Fluid	Turbid			
36.7^{A}	6.7^{B}			**
Exudate colour, %				
Clear	Grey	Yellow		
40.0^{A}	0.0^{B}	3.3^{B}		***
Hemorrages localization, %				
Cranial	Caudal	Longitudinal		
23.3^{A}	0.0^{B}	6.7^{AB}		**
Hemorrages width, %				
Pinpoint	3-5 mm	≥5 mm		
23.3^{A}	6.7^{AB}	0.0^{B}		**

**: P<0.01; ***: P<0.001

At histological level, fibres of WB samples were identified for their larger CSA (P<0.001) and for the higher incidence of giant-type fibres among them (P<0.01) (Table 5). This fact lowered the fibres average number visualized in the field area (80 *vs*. 105; P<0.001). It is worthy to be noticed that giant-type fibres belonging to WB samples possessed CSA values that were far greater than those of the giant-type fibres of the non-affected counterparts (6070 *vs*. 3816 μm^2; P<0.01). The occurrence of giant-type fibres and of fibres with an increased size are indexes of abnormality in muscle architecture and are typical of the degenerative process which establish itself in breast muscle of fast growing and higher yield birds *(Petracci et al., 2013b)*.

Table 5. Histological traits

	N	WB	Significance	RSD[(1)]
Average fibres number (on total field area)	105	80	***	14.7
Fibres CSA, % total field area (HE staining)[(2)]	71.0	73.5	ns	4.5
Fibres CSA, % total field area (Masson's trichrome staining)	67.9	68.1	ns	6.2
Fibre CSA, μm^2	2012	2750	***	332
Giant fibre CSA, μm^2	3816	6070	**	2111
Giant fibres, % total fibres number	1.1	2.0	**	0.8
Giant fibres area, % total field area	2.1	3.8	**	1.6
Collagen area, % total fibres area	16.1	20.0	ns	7.2
Collagen area, % total field area	10.7	12.9	ns	3.4
Empty spaces, % total field area	21.4	19.0	ns	4.5

**: P<0.01; ***: P<0.001; [(1)] Residual Standard Deviation; [(2)]Hematoxylin-Eosin (HE) staining

Contrary to the expectations, but likely due to the limited sampling size, in the present study the pathological condition did not entail an enhancement concerning collagen percentages, and only numerically higher values were observed. Nevertheless, it remains undeniable that collagen proliferation is a typifying characteristic *(Sihvo et al., 2014)*.

Conclusion

Wooden Breast myodegeneration worsens meat quality traits and the visual appearance of the affected breasts through the presence of characterizing lesions. Etiology is unknown at this day, but considering the analogies with WS condition, the high body weight and the superior breast yield reached by birds within a short period could contribute to the myopathy.

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Uticaj "drvenih grudi" na kvalitet živinskog mesa, histološka svojstva i karakterizaciju lezija

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Rezime

Cilj ove studije je da se ispita efekat miodegradacije odn. tzv. drvenih grudi - Wooden Breast (WB) na kvalitet mesa peradi i da se doprinese morfologiji lezija. U objektu za rasecanje živinskog mesa, odabrano je 30 normalnih (N) i 30 fileta grudi pogođenih ovom pojavom (WB) (*pectoralis major*). Težina, poprečni presek (CSA), pH vrednosti L*a*b* vrednosti boja, kapacitet zadržavanja vode i sila presecanja po Warner-Bratzler-u su određivani. Uzorci su i vizuelno i histološki vrednovani. Uzorci pogođeni ovom pojavom su bili teži, deblji, svetliji ($P<0,001$) i odlikuju se palpatornom tvrdoćom i manjom sposobnošću zadržavanja vode ($P<0,05$). Makroskopski, abnormalnosti su, pre svega, lokalizovane u kranijalnom delu filea i definisana prisustvom izbočina, petehijama, tečnosti i eksudata i višim pH. Mikroskopski, WB pojava odn. stanje karakterišu ćelije sa većom CSA ($P<0,001$) i veću rasprostranjenost džinovskih vlakana ($P<0,01$). Podaci ukazuju na postojanje odnosa između težine grudi i WB stanja.

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LIPID COMPOSITION OF MEAT IN MALE LAYER-TYPE CHICKENS AT DIFFERENT AGE

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Invited paper

Astract: An experiment was carried out with male layer-type chickens at the age of 5 and 12 weeks to study the changes in the intramuscular lipid content, total cholesterol and fatty acid composition in breast and thigh. In both muscles the content of total lipids and cholesterol decreased as age increased, and the same was found for the levels of C14:0 and C16:1 ($P<0.001$). Breast muscle in the chickens slaughtered at 12 weeks of age displayed lower content of C18:2 ($P<0.001$), C20:3 ($P<0.01$) and MUFA ($P<0.05$), but higher of C16:0 ($P<0.05$); C20:4 ($P<0.001$), C22:5 ($P<0.001$), C22:6 ($P<0.05$) and the total amount of n-3 PUFA ($P<0.05$). In thigh muscles the older age led to lower levels of C18:0 ($P<0.05$), C18:3 ($P<0.001$) and C20:3 ($P<0.001$) while C20:4 and C22:5 increased significantly ($P<0.001$). No changes in the ratio PUFA/SFA in breast and thigh due to the age of the birds was found, however it remained higher than the recommended minimal values.

Key words: layer-type chickens, meat, cholesterol, fatty acids, age

Introduction

The studies on the possibilities to utilize the male layer-type chickens started in the mid-70s. So far the methods applied contradict the principles of animal welfare. Often, the one day-old male layer-type chickens have been used in the pet food industry as a high quality animal protein source, or they have been killed due to their poor fattening performance and consequently high fattening costs. Moreover the male layer-type chickens have not been accepted as birds for meat. During the last decade the meat of slow-growing broilers, reared in organic systems began to draw the attention as a high quality and healthy product despite its high price (*Zelenka et al., 2001; Lonergan et al., 2003; Lichovníková et al., 2009).* According to several authors, the male layer-type chickens respond entirely to all the requirements of the slow-growing broilers and at the same time are adapted to the alternatve rearing systems.

Fat is an important component of the human diet, however its high intake levels are associated with increased risk of cardiovascular diseases, obesity and diabetes. Most current dietary guidelines *(FAO, 2010)* recommend limiting daily fat intake to maximum 30-35% of total energy, as SFA and *trans* fatty acids contributing no more than 10%. Many consumers believe that red meat is not healthy due to the relatively high SFA and cholesterol contents. It has been recently demonstrated that replacement of red meat with chicken is associated with a significant decrease in apolipoprotein B and total cholesterol levels in micro albuminuric type 2 diabetic patients *(Gross et al., 2002)*. This effect is probably related to the higher PUFA content of chicken meat in comparison to beef. The lipid composition of meat is mostly affected by nutrition of the birds, but other factors such as age and sex also contribute to the changes in the fatty acid profile. The aim of this work was to compare the lipid composition of male layer –type chickens at different age.

Materials and Methods

The experiment was carried out with 150 male layer-type chickens. They were fed a diet with a composition as presented on Table 1.

Table 1. Diet composition

Feed	Protein, %	Fat, %	Dry matter, %	Ashes, %	NNC, %
Starter	20.26	2.81	3.39	4.60	56.11
Grower	18.51	6.39	4.36	4.48	56.22
Finisher	19.13	6.38	5.33	3.90	56.51

At the age of 5 and 12 weeks 10 chickens were slaughtered. The carcasses were kept at 4°C for 24 hours. Breast and thighs of each chicken were separated, minced with meat grinder, and samples (10 g) for lipid analysis were taken. Total lipids of the breast and thigh were extracted according to the method of *Bligh and Dyer (1959).* Aliquots of the lipid extract were subjected to determination of total cholesterol content *(Sperry and Webb, 1950).* Methyl esters of the total lipids, isolated by preparative TLC were obtained using 0.01 % solution of sulphuric acid in dry methanol for 14 h, as described by *Christie (1973).* The fatty acid composition of total lipids was determined by GLC analysis using chromatograph C Si 200 equipped with capillary column (TR-FAME - 60 m x 0.25 mm x 0.25 μm) and hydrogen as a carrier gas. The oven temperature was first set at 160 °C for 0.2 min, then raised until 220 °C at a rate of 5 °C/min and hold for 5 minutes. The temperatures of the detector and injector were 230 °C. Methyl esters are identified

comparing to the retention times of the standards. Fatty acids are presented as percentages of the total amount of the methyl esters identified (*Christie, 1973*).

Data were statistically analysed by t-test using JMP v.7 software package, at levels of significance: * P<0.05; **P<0.01; ***P<0.001.

Results and Discussion

The total lipid content in breast muscle (Table 2) was significantly influenced by the age of the chickens (P<0.01). It was higher in the 5 weeks old birds - 1.03% *vs.* 0.77% in the chickens slaughtered at 12 weeks of age. Similarly the content of lipids in thighs of the younger chickens was substantially higher (P<0.001) when compared to the 12 weeks old birds, ranging from 3.31-1.93 %. The age of the birds affected considerably the total cholesterol content in breast and thighs, showing significantly lower content in the older chickens (P<0.001), corresponding to the lower total lipid contents in these birds. The values varied between 71.83-52.83mg/100g in breast and 100.99-73.36 mg/100g, respectively in the 5 and 12 weeks old chickens. Age decreased the total lipid content by 24% in breast and 42% in thigh muscles, while the cholesterol content was decreased by average of 27 % in both breast and thigh. Studies on the effect of age at slaughter have focused mainly on the sensory parameters of the meat and the data concerning lipids are relatively inconsistent. Similar to us, *Touraille et al. (1981)* found decrease in the lipid content in chicken meat after 8 till 16 weeks of age, while *Zanusso et al. (2002)* reported constant increase in the lipids in breast and thigh from 6 till 22 weeks.

Table 2. Total lipid and cholesterol content in breast and thigh muscles in chickens at different age (mean±SEM)

Item	5 weeks	12 weeks	Significance
Breast			
Total lipids (%)	1.03±0.08	0.77±0.03	**
Cholesterol (mg/100g)	71.83±1.56	52.83±1.42	***
Thigh			
Total lipids (%)	3.31±0.11	1.93±0.12	***
Cholesterol (mg/100g)	100.99±2.25	73.36±3.23	***

The major saturated fatty acids identified in breast (Table 3) and thigh (Table 4) are palmitic (C16:0) and stearic (C18:0). The content of C16:0 in breast varied between 20.35-21.64 %, showing significantly higher content (P<0.05) in the 12 weeks old chicken. In thigh muscles the amount of C16:0 was lower (17.29-17.36%) and was not affected by the age of the chickens. No difference between the two age groups was observed for the content of C18:0 in the breast. Its amounts averaged 9.81 %, while in the thighs it varied between 8.31-7.73% and

was significantly lower (P<0.05) in the older chickens. Myristic acid (C14:0) is the third saturated fatty acid identified in the breast and thigh muscle. Its content was in the range of 0.27-0.19% in breast but higher in thighs (0.39-0.23%). Contrary to C16:0, C14:0 had significantly lower content in the breast (P<0.05) and thighs (P<0.001) in the 12 week old chickens. The content of the saturated fatty acids in the meat of the chickens depend on their presence in the diet as well as their synthesis in the liver *(Sheehy et al., 1993)*. In both breast and thigh the content of the C14:0 remained substantially lower in the older chickens. The same tendency was kept for C18:0, while for C16:0 we did not observe consistent change in both groups. As a whole saturated fatty acids can have considerable influence on the levels of blood cholesterol and LDL cholesterol. However according *Rioux and Legrand (2007)*, the saturated fatty acids differ in their cholesterol rising effect as it is different for fatty acids with medium chain lengths, such as lauric C12:0, myristic C14:0 and palmitic C16:0. Stearic acid does not raise blood cholesterol to the same extent as myristic, lauric and palmitic acids, because it is converted to its mono-unsaturated form (oleic acid C18:1) in the liver very efficiently. As a whole poultry meat has lower content of C14:0 when compared to the other species and in particular with ruminant animals. The levels of this fatty acid determined in our study are approximately two times lower than those reported by *Rule et al. (2002)* and *Cantor et al. (2008)* in broilers, which could indicate certain advantage of the male layer-type chickens and particularly those slaughtered at older age as a source of healthy meat.

Table 3. Fatty acid composition of breast of the chickens, slaughtered at 5 and 12 weeks of age

Fatty acid, %	5 weeks	12 weeks	Significance
C14:0	0.27±0.02	0.19±0.02	*
C16:0	20.35±0.29	21.64±0.45	*
C16:1	1.33±0.19	0.43±0.10	***
C18:0	10.01±0.41	9.61±0.23	NS
C18:1	26.93±1.09	25.06±0.63	NS
C18:2	29.79±0.37	26.48±0.62	***
C18:3	0.30±0.02	0.25±0.01	*
C20:2	0.54±0.03	0.45±0.03	NS
C20:3	0.6±0.04	0.52±0.01	**
C20:4	9.07±0.83	14.37±0.56	***
C22:5	0.30±0.02	0.48±0.01	***
C22:6	0.39±0.04	0.49±0.02	*
SFA	30.64±0.57	31.45±0.67	NS
MUFA	28.27±1.25	25.49±0.72	*
PUFA	41.09±0.92	43.05±0.99	NS
n-6	40.10±0.88	41.83±0.99	NS
n-3	0.99±0.04	1.22±0.02	***
P/S	1.34±0.03	1.37±0.05	NS

Tabel 4. Fatty acid composition of thigh of the chickens, slaughtered at 5 and 12 weeks of age

Fatty acid, %	5 weeks	12 weeks	Significance
C14:0	0.39±0.01	0.23±0.01	***
C16:0	17.29±0.19	17.36±0.36	NS
C16:1	2.78±0.17	1.03±0.12	***
C18:0	8.31±0.19	7.73±0.14	*
C18:1	31.15±0.63	32.48±0.36	*
C18:2	33.84±0.57	33.53±0.54	NS
C18:3	0.49±0.01	0.41±0.01	***
C20:2	0.30±0.01	0.28±0.01	NS
C20:3	0.26±0.01	0.19±0.01	***
C20:4	4.81±0.22	6.32±0.29	***
C22:5	0.15±0.01	0.21±0.01	***
C22:6	0.20±0.01	0.20±0.01	NS
SFA	25.99±0.34	25.32±0.43	NS
MUFA	33.94±0.75	33.51±0.41	NS
PUFA	40.06±0.73	41.15±0.73	NS
n-6	39.22±0.71	40.33±0.71	NS
n-3	0.85±0.02	0.82±0.02	NS
P/S	1.54±0.03	1.63±0.05	NS

The fatty acid which formed the higher percentage of the monounsaturated fatty acids in both muscles was oleic (C18:1). Its content varied within the range of 26.93-25.06% and 31.15-32.48% respectively in the breast and thigh, as in the latter it showed higher content in the older chickens ($P<0.05$). Significant difference between the groups was observed in regards to the content of the other monounsaturated fatty acid – C16:1, which displayed substantially lower levels in the 12 week old chickens ($P<0.001$) in both breast and thigh. The increase in the content of the oleic acid in the thigh muscles corresponds to the decrease of the stearic acid in the same muscles of the chickens at 12 weeks of age.

Linoleic acid (C18:2) is the most abundant polyunsaturated fatty acid. In breast its amounts were 29.79-26.48% and was significantly lower in the chickens slaughtered at 12 weeks of age ($P<0.001$). In thigh muscle the content was slightly higher (33.84-33.53%) but no difference in the two groups was observed. The other essential polyunsaturated fatty acid – linolenic (C18:3) showed average content of 0.28% and 0.45% respectively in the breast and thighs. It was affected by the age of slaughter, showing lower contents in the 12 week old chickens. Both linoleic and linolenic acids are precursors for the synthesis of the fatty acids of n-6 and n-3 families, including respectively arachidonic as well as docosapentaenoic and docosahexaenoic fatty acids. In both breast and thighs C20:4 showed substantial increase in the older chickens ($P<0.001$). Its amounts were relatively high in both muscles – 9.07-14.37% and 4.81-6.32% respectively in breast and thigh. Similar to

C20:4, eicosapentaenoic acid (C22:5) showed significantly higher content (P< 0.001) in the two examined muscles of the chickens at the age of 12 weeks. In breast its content varied between 0.30-0.48%, while in thighs it was lower (0.15-0.21%). No difference in the content of C22:6 was observed between the two groups. The older age of slaughter led to significantly lower content of C20:3 in both muscles (P<0.01; 0.001).

Contrary to most of the individual fatty acids, differences between the two age groups in the total amounts of the fatty acids were observed only in regards to monounsaturated and n-3 polyunsaturated fatty acids in breast. The total content of MUFA was significantly lower (P<0.05) and that of total n-3 PUFA was higher (P<0.001) in the older chickens. These changes corresponded to those found for the individual mono and n-3 polyunsaturated fatty acids in the breast muscle. On the other hand the lack of significant differences in the total amounts of monounsaturated fatty acids in the thighs as well as saturated and polyunsaturated fatty acids in both muscles are due to the diverse tendencies of change in the individual fatty acids between the two age groups. Meat has been implicated in imbalanced fatty acid intake by consumers, due to some meats naturally having a low PUFA to SFA ratio (P/S) ratio of around 0.1. Thus a P/S ratio of 0.4 is often recommended *(Wood et al., 2003, Grashorn, 2007)*. Also, it is recommended that dietary energy coming from fat for human consumption should not be more than 30%, while the relation among SFA, MUFA and PUFA should equal 1:1:1 *(Raats et al., 2009)*. In the current study although not significantly different among groups, the P/S ratio was higher than 0.4, ranging between to 1.34-1.38 in breast muscle versus 1.63 to 1.54 in thighs.

Conclusion

The content of total lipids, cholesterol and hypercholesterolemic C14:0 decreased between 5 and 12 weeks of age in breast and thigh muscles in the male layer-type chickens. Breast muscle displayed lower content of C16:1, C18:2, C20:3 and MUFA, but higher of C 16:0; C20:4, C22:5 and C22:6 and the total amount of n-3 PUFA in the chickens slaughtered at the age of 12 weeks. In thigh muscles the older age led to lower levels of C16:1, C18:0, C18:3 and C20:3 while C20:4 and C22:5 increased. In both muscles the ratio PUFA/SFA was not influenced by the age of the chickens, but remained higher than the recommended minimal values.

Sastav lipida mesa u muških pilića različitog uzrasta

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Rezime

Eksperiment je izveden sa muški pilićima u uzrastu od 5 i 12 nedelja sa ciljem da se ispitaju promene intramuskularnog sadržaja lipida, ukupnog holesterola i sastava masnih kiselina u grudima i karabatacima. U oba mišića sadržaj ukupnih masti i holesterola je opao sa starošću, a isto je utvrđeno za nivoe C14:0 i C16:1 (P<0,001). Mišići grudi u pilića zaklanih u 12 nedelja starosti pokazuju niži sadržaj C18:2 (P<0,001), C20:3 (P<0,01) i MUFA (P<0,05), ali veći sadržaj C16:0 (P<0,05); C20:4 (P<0,001), C22:5 (P<0,001), C22:6 (P<0,05) i ukupna količina n-3 PUFA (P<0,05). U mišićima bataka stariji uzrast je dovelo do nižih nivoa C18:0 (P<0,05), C18:3 (P<0,001) i C20:3 (P<0.001), dok su nivoi C20:4 i C22:5 značajno porasli (P<0,001). Nema promena u odnosu PUFA/SFA u grudima i karabatacima zbog starosti ptica, međutim, on je osta veći od preporučenih minimalnih vrednosti.

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THE EFFECT OF RAW SOYBEAN IN THE FINAL MIXTURES FOR BROILER CHICKENS ON THE PERFORMANCE AND CARCASS YIELD

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Invited paper

Abstract: The aim of this study was to point out the possibility of replacing a portion of thermally treated soybean of standard variety "Lydia" and variety with reduced trypsin inhibitor "Lana" with raw grain in the final mixes for broiler chickens. The trial was conducted in the last week of the experiment (age 35-42 day). At the beginning (day 35) and at the end of the experimental period (42 day) body mass of chickens, the average daily gain, feed conversion and consumption were determined. At the end of the trial, a random sample of 12 chickens from each group was taken (6 male and 6 female) in order to obtain dressing percentage indicators. The results showed that the increased concentration of TI in mixtures caused significant decrease of average daily gain. Consumption of food was not influenced by the studied factors. Feed conversion was significantly better in chickens fed diets containing soy with lower TI. The share of raw soybean in diets of 15 and 20% resulted in a significantly lower feed conversion. Carcass yields of broiler chickens were significantly influenced by the level of raw soybean in the mixture. The share of abdominal fat was not influenced by the studied factors.

Key words: broiler chickens, soybean, trypsin inhibitor, production performance, carcass yield

Introduction

Soybean is the most important protein component in poultry nutrition. The anti-nutritive substances present in raw soybeans reduce the thermal treatment. As a result of the use of raw soybeans in poultry nutrition products depression in performance results occurs *Douglas et al. (1999).* The presence of anti-nutritional

substances in soybeans can be reduced through selection and breeding, creating varieties with reduced content of some anti-nutritive matters. In our agroecological conditions, variety Lana has been created with lower levels of trypsin inhibitor.

Comparing the nutritional value of soybean varieties with lower TI in experiments conducted on chickens *(Han et al., 1991; Jokić et al., 2004; Beuković et al, 2010a; Petričević et al., 2013)*, hens *(Zhang et al., 1991; Petričević et al., 2014a; Petričević et al., 2014b)* and pigs and chickens *(Palacios et al., 2004)* authors have reported better results compared to varieties with a standard level of TI.

The objective of this research was to investigate the effects of replacing a part of thermally processed soybean of variety with a standard level of TI and varietiy with reduced TI, with raw soybean grain of said varieties in the final mixtures for broiler chickens on performance, carcass yield and share of abdominal fat. By determining the replacement level of heat treated soybean with raw soy grain, which has no negative impact on the performance and the quality of the product, a significant reduction in production costs can be achieved resulting in the improved economical efficiency.

Materials and methods

The research was conducted at the experimental farm of the Institute for Animal Husbandry in Zemun on broiler chickens of heavy line hybrid Hubbard F15. In the nutrition of boilers, in the final mixtures, two local varieties of soybean were used - "Lana" with reduced trypsin inhibitor and standard variety "Lydia", thermally treated and raw. The effect of different levels of raw soybeans in diets on the performance of broiler chickens and yields was determined by two factorial experiment 2 x 5 (two soybean varieties x 5 levels of participation of the raw grain in mixture) with 10 nutrition treatments. 2000 one day old chicks were divided into 40 boxes, so there were four replicates per treatment.

By the 35th day of the experiment, all broilers were fed the same feed mixtures according the recommendations for the studied hybrid. Finisher as the final mixture, containing 17.5% crude protein and 13.2 MJ/kg metabolizable energy, was available to broiler chicks from 35 to 42 days, and differed in all the tested treatments in the soybean varieties used and the participation of thermally treated and raw soybean. The share of thermally processed grain of both soybean varieties accounted for 20% of the mixture and was included in the two control treatments (K). In the groups (I), 15% of the total of 20% of full-fat soybean in the mixture was heat-treated and 5% of soybean was raw. In groups (II), 10% of heat-treated and 10% of raw soybean was added to the mixture. In the groups (III), of

20% of full-fat soybean grains in the mixture, 5% was thermally treated and 15% was raw. In groups (IV), 20% of the raw soybeans was incorporated in the diet.

Measurement of body weight of chickens was carried out at the beginning (day 35), and at the end of the experiment (day 42). Based on the difference in body weight of chickens the average daily gain was calculated. Control of food consumption included also measuring of the amount of complete mixtures given to broiler chicks and the rest of the food for each box. On the basis of data on food consumption and gain of chickens, the feed conversion ratio was calculated.

At the end of the experiment, a sample of 6 broiler for each studied treatment and sex was randomly selected, a total of 120 chickens. After a fasting period of 12 hours, body weights were measured and subsequently chickens were slaughtered. Post slaughter, carcasses were chilled to 4°C for 24 hours and then processed in accordance with the *Rulebook on quality of poultry meat (Pravilnik o kvalitetu mesa, 1981).* In this way the following carcasses were obtained: - "conventional dressing" – carcass with head, neck, legs and edible internal organs (heart, lungs, liver, gizzard, kidneys and spleen); – "ready to roast" – carcass without head and legs but with neck and edible internal organs; - "ready to grill" – carcass without head, legs, neck and edible internal organs. In processing of the carcasses the abdominal fat was separated. The resulting weights of carcass and abdominal fat were placed in relation to pre sluaghter body weight of chickens. In this way, the carcass yields and the share of abdominal fat in the carcass were obtained.

For statistical analysis the software package STATISTICA, version we used 6 (StatSoftInc.). The level of statistical significance of differences between groups was determined using the Tukey test.

Results and discussion

Table 1 shows the production performance traits of broilers. Chickens fed diets containing soybean with lower TI had a higher average gain at the age from 35 - 42 days, compared to chickens fed a diet containing soybean with a standard level of TI, where established differences were not statistically significant. With increasing levels of raw soybeans in diets for chickens in the final stage of fattening, the average gain statistically significantly ($p<0.05$) decreased. Interaction of studied factors had no significant effect on this production indicator. Consumption of food of broiler chickens showed no significant differences under the influence of varieties and the level of raw soybeans in the mixture.

Table 1. Production performance

Treatment	Daily gain, g	Feed consumption, g	Feed conversion, kg
	35-42 day		
Soybean variety			
Lana	76.97	196.96	2.57[b]
Lydia	73.92	202.62	2.77[a]
Level of raw soybean, %			
0 (K)	82.37[a]	194.4	2.36[c]
5 (I)	80.86[a]	198.51	2.45[bc]
10 (II)	76.18[ab]	205.17	2.70[ab]
15 (III)	70.50[b]	199.65	2.84[a]
20 (IV)	67.32[b]	201.22	3.00[a]
p value			
Soybean variety	ns	ns	0.001
Level of raw soybean	0.001	ns	0.001
Variety x Level	ns	ns	0.001

a-b Average values in each column with no common designation are significantly different at the level of 5%

Palacios et al. (2004) have reported significantly lower ($p<0.05$) values of the average gain of chickens fed diets containing raw soybeans compared to chickens fed diets based on soybean meal. *Jokić et al. (2004)* have determined a significantly lower gain of chickens fed diets containing the raw standard soybean variety, compared to mixtures based on heat-treated soybean. *Loeffler (2012)* found no statistically significant effect of increasing concentrations of TI in mixtures for broiler chickens on feed consumption which is consistent with the results obtained in our study. Also, *Anderson-Hafermann et al. (1992)* and *Zhang et al. (1993)* have found significant differences in feed intake of chicks fed diets containing soybean with different levels of TI. *Douglas et al. (1999)* report that chickens fed diets containing raw soybean with a standard level of TI consumed the same amount of food as chickens fed diets containing soybean with lower TI and soybean with lower levels of lectins. Contrary to these results *Sardary (2009)* states significantly lower feed consumption of broiler chickens that have used a mixture with 20% share of raw soybeans compared to the control group that consumed the mixture without the participation of raw soybean grains.

Using soybeans with a standard level of TI in the final mixes for feeding of broiler chickens resulted in the significantly lower feed conversion ratio ($p<0.05$) compared to chickens fed diets containing soybean with reduced levels of TI (Table 1). Differences in feed conversion were statistically significant under the influence of the level of raw soybean in mixtures. The best feed conversion is recorded in chickens that consumed mixtures without raw soybean. With the increase in the share of raw soybean in the final mixtures for broiler chickens, feed

conversion ratio was worse. The interaction of investigated factors resulted in statistically significant differences ($p<0.05$). The worst feed conversion ratio was recorded in group of chickens with maximum share of raw soybeans.

Rand et al. (1996) have found poorer feed conversion ratio of chickens fed diets with the raw soybean share of 20% (2.65 kg) compared to chickens fed diets containing the same amount of soy grits (2.38 kg). *Beuković et al. (2010b)* also points out the negative effect of the use of raw standard soybeans in chicken nutrition in the sixth week of the experiment (2.73 kg) compared to raw soybeans with lower TI level (2.31 kg). A higher concentration of TI in mixtures for broiler chickens caused a significantly poorer feed conversion according to *Palacios et al. (2004)*. Data on feed conversion in our study are consistent with the results which were obtained by *Douglas et al. (1999)* and *Sardary (2009).*

Table 2 shows the yields of broiler chickens and share of abdominal fat in the carcass. A ratio between the absolute values of the yield of dressed carcasses and pre-slaughter body weight allows for objective examination of the influence of the studied factors. Carcass yields of broiler chickens were not significantly influenced by soybean varieties. Chickens fed diets containing a higher percentage of raw soybeans had significantly lower ($p<0.05$) yield values. Interaction of investigated factors reulted in significant differences in average values for yield.

Table 2. Carcass yield and share of abdominal fat

	Carcass yield			Share of
Treatment	Conventional	Ready to	Ready to	abdominal
	dressing, %	roast, %	grill, %	fat, %
Soybean variety				
Lana	82,7	76,4	67,7	0,80
Lydia	83,1	76,8	68,2	0,78
Level of raw soybean, %				
0 (K)	83,6[a]	77,2[a]	68,9[a]	0,75
5 (I)	83,4[a]	77,2[a]	68,4[ab]	0,83
10 (II)	82,5[b]	76,3[b]	67,5[ab]	0,82
15 (III)	82,4[b]	76,2[b]	67,7[ab]	0,84
20 (IV)	82,5[b]	76,0[b]	67,3[b]	0,70
p value				
Soybean variety	ns	ns	ns	ns
Level of raw soybean	0,022	0,035	0,012	ns
Variety x Level	ns	ns	ns	ns

* a-b Average values in each column with no common designation are significantly different at the level of 5%

In experiments conducted on broiler chickens of genotype Hubbard *(Petričević et al., 2011; Petričević et al., 2012)* similar values of all three dressing percentages were found, same as in this study. *Subuh et al. (2002)* found no significant differences in carcass yield of broiler chickens fed diets with different amount of full-fat soybean. *Sardary (2009)* found no statistically significant differences between groups for carcass yield of chickens fed diets containing raw and heat-treated soybean. The author states that the yield ranged from 69.8% to 72.2%.

Abdominal fat is an important indicator of carcass quality of broiler chickens given the high and positive correlation with the total amount of fat in the carcass. Feeding broiler chickens mixtures containing domestic soybean varieties with different levels of TI had no statistically significant impact on changes in the share of abdominal fat. The analysis of the impact of the level of raw soybeans in diets showed no statistically significant differences between groups in the share of abdominal fat. Also, the interaction of investigated factors did not result in the significant differences in the share of abdominal fat (Table 2).

Beuković et al. (2012) found a statistically significantly lower share of abdominal fat (0.76%), when using raw soybean in the diet of chickens, compared to the share of abdominal fat of chicks fed diets containing heat-treated soybean (1.04%).

Conclusions

With the reduction of the share of raw soybeans in diets, the average daily gain of chickens increased significantly. Feed consumption was not significantly different under the influence of the examined factors and their interactions. Feed conversion was significantly influenced by soybean varieties and levels of raw soybean in mixtures. Food consumption per unit of gain was significantly better in chickens fed diets containing soybean with lower TI level than the standard soybean variety. With increasing levels of raw soybeans in diets feed conversion worsened.

Carcass yields of broiler chickens were significantly influenced by the level of replacement of heat treated with raw soybean. Carcass yields "conventional dressing" and "ready to roast“ were significantly higher in the group with no raw soybean and groups with 5% of raw soybean in the diet, compared to the group fed diets with 10%, 15% and 20% of raw soybean. In case of "grill ready/ready to grill", the differences were statistically significant between the groups of chickens fed diet without raw soybeans and groups that were fed with a mixture of 20% raw soybean.

Mass and share of abdominal fat in carcass of broiler chickens were not significantly influenced by the studied factors.

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Efekat upotrebe sirove soje u završnim smešama za ishranu brojlerskih pilića na proizvodne pokazatelje i randmane

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Rezime

Cilj ovih istraživanja bio je da ukaže na mogućnost zamene jednog dela termički obrađenog sojinog zrna standardne sorte „Lidija“ i sorte sa smanjenim sadržajem tripsin inhibitora „Lana“, sirovim zrnom u završnim smešama za ishranu brojlerskih pilića.

Ogled je izveden u poslednjoj nedelji tova (35-42. dan uzrasta). Tokom istraživanja svakodnevno je evidentirana količina utrošene hrane. Na početku (35. dana) i na kraju oglednog perioda (42. dana) izvršeno je merenje telesnih masa pilića, utvrđivanje prosečnih dnevnih prirasta, konzumacije i konverzije hrane. Na kraju ogleda metodom slučajnog uzorka iz svake grupe žrtvovano je po 12 pilića (6 muških i 6 ženskih) u cilju dobijanja klaničnih pokazatelja.

Dobijeni rezultati su pokazali da povećana koncentracija TI u smešama utiče na značajno smanjenje prosečnih dnevnih prirasta. Konzumacija hrane nije bila pod uticajem ispitivanih faktora. Konverzija hrane je bila statistički značajno bolja kod pilića hranjenih smešama koje sadrže soju sa nižim nivoom TI. Učešće sirove soje u smešama od 15 i 20% uticalo je na značajno lošiju konverziju hranu. Randmani brojlerskih pilića su bili pod značajnim uticajem nivoa sirovog zrna soje u smeši. Udeo abdominalne masti nije bio pod uticajem ispitivanih faktora.

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BIOMASS UTILIZATION SYSTEMS FOR SERBIAN LIVESTOCK FARMERS – POTENTIALS AND REALITY

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Invited paper

Abstract: The paper analyses the availability and energy potential of biomass originating from agriculture and animal husbandry in Serbia, and generally recommends the necessary changes in order to increase the use of biomass as an energy source in Serbia. Also, detailed analysis of a simple example of the possible applications of biomass to livestock farms is presented.

Key words: biomass, energy, livestock farms, Serbia

Introduction

Three key resources to focus on are water, food and energy, and our most important goal and a pledge for the future must be to ensure them in sufficient quantities in a sustainable manner at both the local and global level. Agriculture and livestock are of great importance for the production, consumption and conservation of these three resources (*Lukić et al., 2013*). Serbia is unfortunately already late with the introduction of changes in production methods in order to preserve resources and increase production. It is indicative that despite the great relevance and urgency, there is relatively little published research in this field in our country, especially those relating to the energy aspect of livestock farms.

When we speak of energy and its production and consumption in a sustainable manner in agriculture and animal husbandry, we are really talking about biomass, which is becoming increasingly important and desirable energy source. The fact is that the use of biomass in the production of thermal and/or electrical energy does not adversely affect the environment, it is locally available in agricultural regions and farms and unused, and as such it represents a cost-effective solution (*Yamashiro et al., 2013; Iwasaki et al., 2013*). On the other hand, the use of biomass cannot completely replace other energy sources, but can significantly contribute to reducing energy needs and increase energy security both at local and global levels, while reducing emissions of greenhouse gases.

The aim of this study is to analyze and briefly show the current availability and biomass energy potential in Serbia, and generally recommend the necessary and feasible changes that would increase the use of biomass in Serbia. Also, in details will be presented and a simple example of possible changes in energy sources, or the application of biomass as an energy source in livestock farms analysed.

Biomass as energy resource

There are many definitions of biomass. Basically, biomass is organics, which is produced by the activities of living creatures, and can be used as energy and material. It is therefore understandable why the main sources of biomass in all countries of the world, although not the only sources, agriculture and forestry. Often the production of biomass for energy purposes is identified with these two production as its integral part, which is increasingly gaining in importance.

With the use of biomass as fuel, the three key aspects are to be analysed:

- Resources - assessment of the potential and actual biomass availability in terms of quantity and energy value, taking all factors into account;
- Conversion - choosing the most appropriate process/technology specific use of biomass for energy in the concrete case;
- System - the development, analysis and elaboration of models and concrete project, which should in practice facilitate the applicability, sustainability and cost-effectiveness of the use of biomass as fuel.

If we analyse the biomass as a resource in our country, it is well known that biomass is considered individually the most important potential source of renewable energy in Serbia. It is estimated that biomass represents 55%, and by some estimates up to 64% of the total potential of all renewable energy sources in Serbia. It is also well known that this potential is not nearly enough utilized. Total biomass energy potential in Serbia is estimated at 3.4 Mtoe (wood biomass, 1.5 Mtoe; agricultural biomass, 1.7 Mtoe; biodegradable waste, 0.2 Mtoe) and currently about 1 Mtoe is being used, almost exclusively of wood biomass.

Table 1 shows estimates of the total annual production potential and unexploited biomass in Serbia.

Table 1. Total amount of unused biomass in Serbia (rough estimation)

Biomass type and remarks	Amount (million tons per year)
• Crop byproducts (field remains from cca. 2.3 million ha)	**15 Mt/y**
• Fruit and grapes byproducts (0.35 mil ha, pruning remains)	**0.6 Mt/y**
• Wood byproducts (without firewood)	**1 Mt/y**
• Livestock manure	**20 Mt/y**

Source: Statistical Office of the Republic of Serbia

From the estimated total annual production, it is evident that the greatest untapped potential in Serbia is biomass from agricultural production, primarily from the fields of corn and wheat, extremely dominant field crops in our country. Also, a significant potential in terms of total annual production is manure from livestock production. However, this biomass potential should be accepted with great reserve, due to a number of factors that have a crucial impact on the availability of biomass. Soil fertility protection is a very important issue, as well as livestock production - animal feeding and bedding, mechanization losses in the collecting process, too big collecting and transport costs or other unfavourable economic aspects. Therefore all the biomass potential cannot be used as an energy source. In order to obtain more realistic data on the availability of these resources and their energy levels further analysis and calculations are required, and one of the possible calculations is given in Table 2. The present calculation is based on average annual areas under maize and wheat, and the expected average annual number of cattle, pigs and poultry in Serbia in the future (*Lukić et al., 2009; Lukić, 2012; Lukić et al., 2013).*

Table 2. Potential and availability of main unused biomass in Serbia (estimation)

Crop field remains (corn and wheat)

Corn remains - potential
Yearly production of corn (from 1.2 mil. ha): 6 million tons of grain
Ratio of waste production for corn: 1.0 t/t
(6Mt grain/year)(1.0t/t) = 6Mt corn remains/year
Coefficient of energy conversion for corn: 17.7GJ/t
(6Mt/year)(17.7GJ/t)= 177PJ/year
Corn remains – availability
Availability ratio for energy production in case of agricultural waste (including corn remains and wheat straw) is 25%.

(177PJ/year)(0.25) = **44PJ/year**

Wheat straw - potential
Yearly production of wheat (from 0.5 mil. ha): 2 million tons of grain
Ratio of waste production for wheat: 1.3t/t
(2Mt grain/year)(1.3t/t) = 2.6Mt wheat straw/year
Coefficient of energy conversion for wheat: 17.5GJ/t
(2.6Mt/year)(17.5GJ/t)= 45.5PJ/year
Wheat straw – availability
(45.5PJ/year)(0.25) = **11.4PJ/year**

Livestock manure

Cattle
900.000 heads (number of cattle) x 1.1t/y/head = 1Mt of dry manure per year
(1Mt/year)(15GJ/t) = 15PJ/year (potential)
(15PJ/year)(0.125) = **1.9PJ/year (available)**
Pig
3 million heads (number of pigs) x 0.22t/y/head = 0.7Mt dry manure/year
(0.7Mt/year)(17GJ/t) = 12PJ/year (potential)
(12PJ/year)(0.125) = **1.5PJ/year (available)**
Poultry
23 million (poultry number) x 0.037t/y/head = 0.85Mt dry manure/year
(0.85Mt/year)(13.5GJ/t) = 11.5PJ/year (potential)
(11.5PJ/year)(0.125) = **1.4PJ/year (available)**

Sources for statistics: Agriculture in Serbia in 2013, Green book, Ministry of Agriculture RS
Source for used calculations coefficients: The Asian Biomass Handbook, Japan Institute of Energy

Because of obviously great potential of biomass in Serbia, currently there are many ongoing international projects, aimed at greater use of biomass in our country and of whom much is expected. The most detailed analysis of the potential and availability of biomass for energy in Serbia, so far, was created and recently released as a part of one of the projects (DBFZ, 2015). However, the availability of significant volumes of biomass in a certain area does not necessarily lead to the realization of the objectives of these projects (*Inoue et al., 2009*). In each case, it is necessary to individually carefully and thoroughly consider all three key aspects of using biomass as a fuel, as has already been pointed out in this paper.

Another major disadvantage is the dominance of small farms and landholdings, and biomass is often spread over a large area in relatively small quantities. Thus, in the planning and construction of larger plants and systems it is necessary to ensure a strong and economically cost-effective logistic support for the collection of biomass and its storage. At the same time, in Serbia, it is important to focus on increasing the use of biomass for energy in small and medium-sized farms, or where it is produced, whereby one should aim at simpler and more cost-effective ways of implementation. Also, in the system using biomass as an energy source, it is necessary to ensure continuous collection of biomass

during the period of several years and thus ensuring the security of supply of biomass, especially in our conditions where there is still no developed market for biomass.

The analysis of the current situation, i.e. the real potential, needs and opportunities for increased use of biomass in farms in Serbia, can result in practical recommendations to farmers, experts and lawmakers that could serve as a basis for the development of models and concrete systems using biomass as fuel in individual farms. Possible scenario for growth in the use of biomass as an energy source on farms is shown in Table 3.

Table 3. Possible scenario of better use of biomass resource on farms in Serbia

Target: main unused biomass (estimation):
– Corn field remains: 6 mil.t (potential) (25%)=1.5 mil.t (available) – Wheat straw: 2.6 mil.t (potential) (25%)= 0.7 mil.t (available) – Livestock manure (cattle, pig, poultry): 2.5 mil.t dry (12.5%)= 0.3 mil.t (av.) *How to collect: existing mechanization (small farms) and/or big baler machines (middle and big farms) for field residues; small scale digester (middle farms) and plants for biogas production and cogeneration (big farms) for manure.*
Conversion method, products and distributions:
– For field residue: physical (production of pellets or briquettes) and/or combustion (for small farms only combustion in small scale boilers – heat production for households and farms in winter time). Products: pellets or briquettes (small scale production or bigger facilities/plants baying corn or straw big bales from farmers). – For manure: middle farms – anaerobic digestion and heat production for farm self-usage. Big farms: anaerobic digestion for methane/hydrogen production and cogeneration systems for heat and electric power production.
What we need for success:
– A lot of education for farmers and pilot plant/training facility establishment (from bottom to top approach). – A lot of research and investment/subsidies support (from top to bottom approach).

Biomass utilization system on livestock farm – one simple and effective model

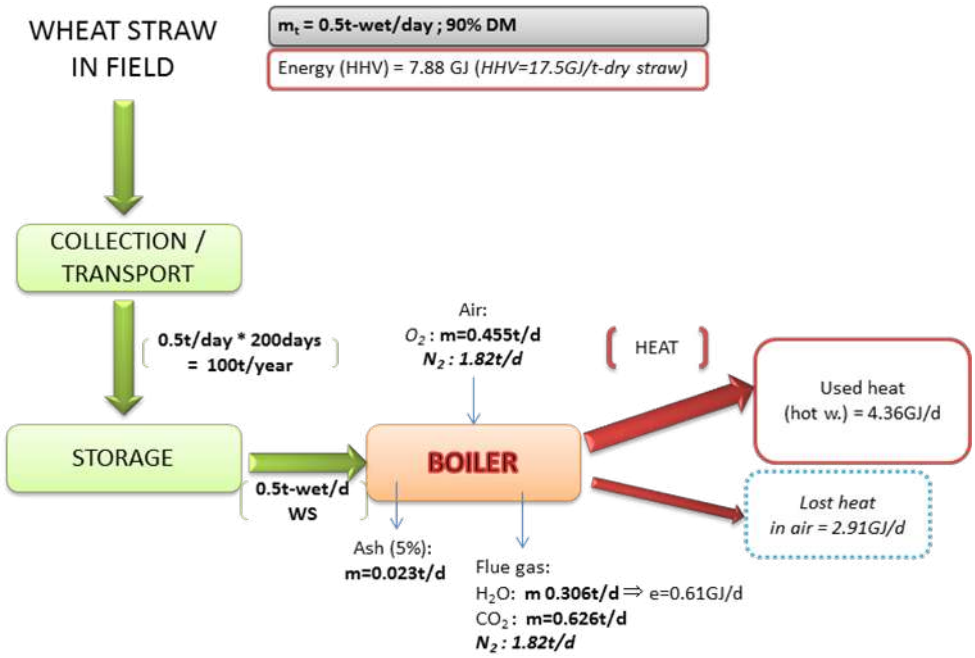
System for using wheat straw for heating pig/poultry farm in winter time (and optional - drying corn grain after harvest)

As an illustration of the possible examples of simple and effective application of biomass as an energy source in pig farms (or poultry), we will consider the effects of substitution of the electric boiler (or fossil fuels) with boiler using the straw as fuel in the existing heating system with hot water. Analysis and development of the system is not made for a real farm, the model and simulation are used, so the results can be used with a certain reserve. Each farm requires a careful consideration of all three key aspects of the use of biomass for energy, including the development and analysis of the relevant final project.

The initial elements for the development and analysis of this example of a very simple system are:

- ***Goal:*** *To develop model of system for using unused wheat straw (or corn remains) from own production which can be replacement for existing heating system on pig farm with electric power boiler (or oil/natural gas boiler).*
- ***Users:*** *Middle scale farms with combined crop and livestock production (with pigs or poultry farm).*
- ***Some boundary and parameters used (suitable) for development/implementation of system:*** *Volume of own production of biomass: ≥ 100t wheat straw per year; Pig farm capacity (for heat consumption): ≥ 200 sows (or broiler farm with equivalent capacity/heat consumption need) with hot water heating system; Heating system running 200 days in year; Collection, transport and labour needs covered by existing farm mechanization and workers.*

A simplified and shortened version of the results of the system analysis shown in this work included three main components of the analysis with graphics and text explanations of the most important indicators. Figure 1 schematically shows the flow of all processes in the planned new system, the use of wheat straw as a fuel, including mass and energy balance of the system.



(Remarks: assumed boiler efficiency 60%)

Graph 1. Flow scheme, mass balance and energy balance of the system

Energy efficiency (Ef) and Energy Profit Ratio (EPR) of the system are 0.55 and 4.36, respectively.

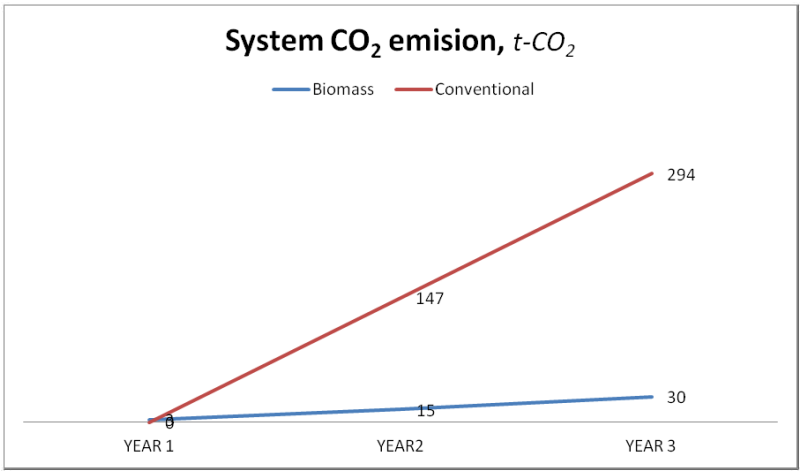
Basic parameters of economic analysis and assessment of the cost-effectiveness of the proposed system in relation to the previous are given in Table 4.

Table 4. Economical evaluation of the system

Cost type, ***way for calculation, formula, explanations …***	**Overall price (€)**
CAPITAL COST ***(estimation based on price of existing similar plants/systems)***	**30,000**
OPERATIONAL COSTS ***(per year):***	
Feedstock *Wheat straw from own production, not purchased. Cost of collecting & transport within farm for 100t wheat straw per year* *(estimate cost for 1t = 20*€)	2,000
Electric power *For running pumps - boiler, hot water heating system* *(100kWh/d *200d*0.05€/kWh)*	1,000
Labor (*covered by existing farm workers)*	0
Maintenance *(estimation, 3% of capital cost)*	900
Unforeseen *(estimation, 1% of capital cost)*	300
OPERATIONAL COST $\sum$ **=**	**4,200**
INCOME (*saving money for electric power needed for pig farm heating per year)* *Calculation: 4.36GJ/day * 200days = 872GJ heat energy per year* *1kWh = 3.6MJ ⇒ 876GJ = 242,000kWh* *242,000kWh * 0.05€/kWh =*	**12,200**

The obtained data indicate that in the market conditions and system parameters used in this example, the annual savings of 8,000 € can be expected with the use of planned new heating system compared to the existing one, and that the investment would be returned in less than 4 years.

Using Life Cycle Assessment (LCA) method, the CO_2 emissions of the old and new systems are established (Graph 2). Using the new system, lower annual CO_2 emissions can be expected by 132.5t, compared to the existing conventional system, i.e. the CO_2 recovery payback time is already in the first year of implementing the new system.



Graph 2. System CO_2 emission, t- CO_2

Finally, it should be noted that already on our farms, especially pig farms, there are very successful examples of the application of such or similar ways of using biomass as fuel. The implementation of such models is expanding mainly due to their simplicity and efficiency, relatively small investments and relatively quick return on investment. The system, depending on the needs and opportunities farms, can also be significantly upgraded and improved.

Conclusion

Serbia has significant energy potential in produced and available biomass, whose efficient use as energy has been current topic for long time, but in reality and practice it is still at the beginning. Considering numerous specificities, primarily large number of small and medium-sized farms that have unused biomass, primarily crop residues and manure, it is important to focus on the increasing application of biomass for energy in these farms, or where it is produced, with tendencies towards more cost-effective ways of implementation. Perhaps in the case of Serbia to plan for increased use of biomass, if we consider the smaller but numerous, as a result we get more.

Acknowledgment

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Sistemi korišćenja biomase za srpske stočare – potencijali i realnost

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Rezime

U radu se analizira raspoloživost i energetski potencijal biomase u Srbiji poreklom iz poljoprivrede i stočarstva i generalno predlažu potrebne promene kojima bi se povećala upotreba biomase kao energenta u Srbiji. Takođe, detaljnije se analizira jednostavan primer moguće primene biomase na stočarskoj farmi.

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MEAT QUALITY AND SENSORY ACCEPTABILITY OF PORK AND FAT FROM ENTIRE MALE, CASTRATED AND FEMALE PIGS

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Abstract: The object of the study was to evaluate pork quality parameters as well as consumer acceptance of meat and fat tissue from entire male (EM) pigs compared to surgical castrates (SC) and gilts (G). Forty-two pigs, each sex of 14, was randomly selected for the experiment. Pigs were crosses of Landrace sows and YxL boars. From seven litters was always selected 6 sibs (2 EM, 2 SC and 2 G). After reaching the average slaughter weight of 105 kg, pigs were slaughtered at the experimental slaughter house. The most of pork quality traits observed did not show any significant differences between sexes. However, three parameters were statistically different between groups of pigs – pH measured 45 min after slaughter in *semimembranosus* muscle (msm), electrical conductivity in the same muscle measured 24 h post mortem and drip loss. Gilts had the highest pH_{45} msm and entire males the lowest (6.74 vs 6.48, $P<0.05$). Significant difference ($P<0.05$) in electrical conductivity was found between entire males and castrates (3.27 vs 4.83 μS). Drip loss was significantly lower in castrated males compared to gilts (2.30 vs 3.23 %, $P<0.05$). Considerable differences between entire males compared to castrates or gilts or both them were observed in all sensory properties of meat samples except for juiciness as well as both flavour and odour of fat tissue.

Keywords: pigs, entire males, pork quality, boar taint, consumer acceptability

Introduction

In most European countries male pigs are surgically castrated to avoid boar taint *(Fredriksen et al., 2009)*. However, there is a growing societal pressure towards to stopping the surgical castration without anaesthesia, or even bann of castration as a whole in all member states of the European Union. Therefore, it is

timely to consider the practical consequences of producing entire male pigs as opposed to castrates. If rearing entire males becomes a prevailing practice in the EU, a risk of boar taint of carcasses from entire males will be increasing.

The two main substances responsible for boar taint are androstenone and skatole. Androstenone (5α-androst-16-ene-3-one) is the steroid acting in sexually maturing boars as a pheromone *(Patterson, 1968)*. It is produced in the testes and found in plasma in free and sulfoconjugated form. Subsequently, it is transported to the salivary glands and deposited to fat tissue. The smell of androstenone is often described as urine-like. It is well known that the ability to detect this smell very varies. A certain part of human population is insensitive – anosmic to the smell of androstenone. This anosmia is genetically determined *(Wysocki and Beauchamp, 1984)* and depends on the sex of consumers and the country of evaluation *(Gilbert and Wysocki, 1987)*. Generally, women are more sensitive to androstenone than men. The percentage of anosmic women with respect to anosmic men was 15.8 vs 24.1 % in Europe (except United Kingdom), 10.9 vs 30 % in UK, 29.5 vs 37.2 % in the USA and 17.2 vs 25.5 % in Asia *(Gilbert and Wysocki, 1987)*. In Germany the percentage of anosmic men compared to women was found to be 70 and 66 %, respectively, and in Spain 60 vs 48 %, respectively *(Weiler et al., 2000)*. Sensitivity of consumers to androstenone has been proved to be an important factor in the consumer acceptability of pork from entire males *(Weiler et al., 2000)*.

Skatole (3-methyl indole) is a breakdown product of microbial degradation of amino acid tryptophan in the hind gut *(Vold, 1970; Walstra and Maarse, 1970)*. The source of tryptophan is the diet and cell debris originated from degradation of intestinal mucosa as well. Similarly as androstenone, skatole is transferred by plasma to adipose tissue. The smell of skatole is reported as faecal-like. While androstenone is not detectable by a part of human population, skatole is perceived by 99 % of the consumers *(Weiler et al., 1997)*. The contributions of androstenone and skatole to boar taint have been investigated in many studies. According to *Berg et al. (1993)*, both compounds make similar contributions to boar taint. A lot of studies *(Lundström et al., 1984; Mortensen and Sorensen, 1984; Andresen et al., 1993; Bejerholm and Barton-Gade, 1993)* reported that skatole had higher contribution to boar taint than androstenone. On the other hand, *Bonneau et al. (1992)* suggested that androstenone contribution was larger than that of skatole. Finally, some authors *(Lundström et al., 1980; Bonneau et al., 1992)* documented that both compounds may have a synergistic effect on boar taint.

The threshold levels used for sensory evaluation of boar taint compounds – androstenone and skatole are usually 0.5 to 1.0 ppm and 0.20 to 0.25 ppm, respectively (as reviewed by *Walstra et al., 1999*). *Font* I *Furnols et al. (2000)* found that in Spain 38 % of the carcasses had high androstenone levels (>1.0 μg/g),

26 % had high skatole levels (>0.22 μg/g) and 17 % had high levels of both compounds.

In addition to the problem related to boar taint in meat from uncastrated males, some studies *(D´Souza and Mullan, 2003; EFSA, 2004)* suggest inferior meat quality of entire males compared to gilts and castrates, especially higher proportion of DFD meat, reduced flavour due to the higher leanness of carcasses from boars. *Font I Furnols et al. (2009)* found less juiciness in pork from entire males than in castrated males and gilts. On the other hand, *Miyahara et al. (2004)* reported that meat from boars was redder and had better ability to retain moisture.

The aim of the presented study was to assess the pork quality of entire males, castrates and gilts of slovak pig population and to find the level of acceptability of pork and adipose tissue from entire males by the slovak consumers.

Material and Methods

Forty-two pigs, entire males (EM), surgical castrates (SC) and gilts (G), each of 14, was randomly selected for the experiment. Pigs were crosses of Landrace sows and YxL boars. From seven litters was always selected 6 sibs (2 EM, 2 SC and 2 G). They were housed in test station at 22-26 kg live weight because of acclimation to new space and feed. Pigs were housed in pairs in pen according to gender.

After reaching the average slaughter weight of 105 kg, pigs were slaughtered at the experimental slaughter house of the Research Institute for Animal Production situated approximately 200 m from the test stable. During the experiment, two pigs (1 EM and 1 SC) were excluded because of health reasons.

A slaughter was done according to standard procedure e.g. electrical stunning, vertical exsanguination, vapor scalding and evisceration. Forty-five minutes after slaughter the electrical conductivity and pH in *musculus longissimus dorsi* (mld) and *semimembranosus* (msm) were measured using device BIOTECH and METTLER TOLEDO with combined electrode, respectively. After that, carcasses were chilled 24 hours at air temperature of 2 °C to 4 °C, air velocity 0.5 to 1.0 m/s started approximately 60 min post mortem. The second day after slaughter, the dissection of the right half of carcasses was done and samples from *longissimus dorsi* muscle (approx. 400 g) and from adipose tissue (over the neck, approx. 200 g) were taken for analyses of pork quality traits and sensory evaluation. Analyses were performed in the laboratory of NAFC – RIAP Nitra. Meat colour was analysed by spectrometry using MINISCAN XE Plus. Water holding capacity (WHC) was analysed using method of Grau-Hamm modified by Hašek and Palanská (1976). Drip loss of meat was determined by method of *Honikel (1998)*. Samples of fat tissue were transported to the authorized laboratory

EKOLAB (Košice, Slovakia) for analyses of androstenone and skatole. Concentrations of these compounds of boar taint were done according to methods described in works of *Ampuero Kragten et al. (2011)* and *Bekaert et al. (2012).*

Four days after slaughter, cooking loss and shear force of meat were determined. Shear force – firmness was measured using TEXTURE ANALYSER TA-XT2i device. Before cooking, 2.0 cm thick slices of *m. longissimus dorsi* were cutting. After that they were cooking on electric contact grill PM-1015 for 4 min at t = 200 °C and cooking loss was determined as difference between weight of meat before and after cooking. Consequently the sensory properties were evaluated. Thirty consumers scored the meat samples for odour, flavou, juiciness, tenderness and overall perception and fat samples for odour and flavour, respectively. Every sample was evaluated by four persons using 5-point scale where 1 – the worst and 5 – the best quality.

Statistical package *SAS (2009)* was employed in the analyses. Basic statistics was done using MEANS procedure. The differences between sexes were analysed using ANOVA:

$$y_i = \mu + B_i + e_i$$

where y_i – characteristic of trait selected
μ – intercept
B_i – effect of sex (i = EM, SC, G)
e_i – random error

Results

Pigs were slaughtered at average slaughter weight of 105 kg (Table 1). Age of entire males at slaughter was significantly lower ($P<0.05$) than that of castrates and gilts (159 vs 171 and 172 days).

Meat quality parameters of entire males, surgical castrates and gilts are shown in Table 2. The most of traits observed did not show any significant differences between sexes. However, three parameters were statistically different between groups of pigs – pH measured 45 min after slaughter in *semimembranosus* muscle, electrical conductivity in the same muscle measured 24 h post mortem and drip loss. Gilts had the highest pH_{45} msm and entire males the lowest (6.74 vs 6.48, $P<0.05$). Significant difference ($P<0.05$) in electrical conductivity was found between entire males and castrates (3.27 vs 4.83 μS). Finally, drip loss was significantly lower in castrated males compared to gilts (2.30 vs 3.23 %, $P<0.05$).

Considerable differences between entire males compared to castrates or gilts or both them were observed in almost all sensory properties of meat samples

(Table 3). Consumers evaluated significantly better odour of meat from gilts (P<0.01) and castrates (P<0.05) compared to boars (4.00 and 3.86 vs 3.43). Flavour of pork from entire males had significantly lower value than that from castrates (P<0.05) but not from gilts.

Significant differences in consumer liking of boar meat compared to gilts or castrates were found in tenderness and overall perception, respectivelly (3.41 vs 3.85, P<0.05 and 3.36 vs 3.76, P<0.05).

Consumer´s evaluation of fat samples of three pig sexes is presented in Table 4. Consumer were much more critical at evaluation of fat from entire males than from castrates and gilts. They gave significantly lowest value for fat odour (EM: 2.64 vs SC: 3.29, P<0.01 and G: 3.49, P<0.001) and fat flavour (EM: 2.86 vs SC: 3.51, P<0.01 and G: 3.42, P<0.05).

Concentrations of the two compounds of boar taint in the samples from adipose tissue of entire males, castrates and gilts are shown in the Table 5. Both values were higher for boars than other two sexes (P<0.05).

Table 1. Basic characteristics of entire males, castrates and gilts

Item	EM	SC	G
Slaughter weight, kg	105.57 ± 1.90	106.07 ± 2.59	104.62 ± 2.40
Carcass weight, kg	84.71 ± 1.78	86.96 ± 3.48	85.96 ± 2.85
Age at slaughter, day	159.00 ± 5.00[a]	171.31 ± 10.70[b]	172.38 ± 9.62[b]

EM = entire males, SC = surgical castrates, G = gilts
[a,b] Values with different letters within rows are significantly different (P< 0.05)

Table 2. Pork quality of entire males, surgical castrates and gilts

Item	EM	SC	G
pH_{45} mld	6.26 ± 0.34	6.38 ± 0.36	6.43 ± 0.33
pH_{45} msm	6.48 ± 0.28[a]	6.50 ± 0.24	6.74 ± 0.22[b]
EC_{45} mld, µS	2.54 ± 1.71	2.39 ± 0.62	2.08 ± 0.24
EC_{45} msm, µS	2.46 ± 0.26	2.45 ± 0.44	2.24 ± 0.41
pH_{24} mld	5.78 ± 0.41	5.81 ± 0.33	5.77 ± 0.37
pH_{24} msm	5.74 ± 0.41	5.76 ± 0.30	5.75 ± 0.34
EC_{24} mld, µS	2.50 ± 1.67	2.62 ± 1.59	2.29 ± 1.65
EC_{24} msm, µS	3.27 ± 1.07[a]	4.83 ± 1.49[b]	4.14 ± 1.81
Colour – japanese scale	2.92 ± 0.34	3.00 ± 0.39	3.23 ± 0.33
Colour – L*	51.53 ± 4.70	50.70 ± 4.93	49.36 ± 2.03
a*	0.87 ± 1.08	0.79 ± 0.61	0.57 ± 0.39
b*	8.13 ± 1.19	8.38 ± 1.60	7.44 ± 0.81
WHC, %	35.65 ± 5.10	34.79 ± 5.16	34.33 ± 5.94
Cooking loss, %	15.19 ± 5.74	15.03 ± 5.55	15.36 ± 6.85
Shear force, W-B	1.83 ± 1.61	1.69 ± 1.37	1.46 ± 1.13
Drip loss, %	2.98 ± 1.05	2.30 ± 0.83[a]	3.23 ± 0.77[b]

EM = entire males, SC = surgical castrates, G = gilts, EC – electrical conductivity, WHC – water holding capacity
[a,b] Values with different letters within rows are significantly different (P< 0.05)

Table 3. Sensory evaluation of pork from entire males, surgical castrates and gilts

Item	EM	SC	G
Odour	3.43 ± 1.11^{a}	3.86 ± 0.82^{b}	4.00 ± 0.65^{b}
Flavour	3.45 ± 0.88^{a}	3.83 ± 0.75^{b}	3.79 ± 0.86
Juiciness	3.53 ± 0.86	3.80 ± 0.92	3.55 ± 1.03
Tenderness	3.41 ± 0.84^{a}	3.80 ± 0.96	3.85 ± 0.79^{b}
Overall perception	3.36 ± 0.79^{a}	3.76 ± 0.86^{b}	3.72 ± 0.74

EM = entire males, SC = surgical castrates, G = gilts
[a,b] Values with different letters within rows are significantly different (min P< 0.05)

Table 4. Sensory evaluation of fat from entire males, surgical castrates and gilts

Item	EM	SC	G
Odour	2.64 ± 1.13^{a}	3.29 ± 1.25^{b}	3.49 ± 0.97^{b}
Flavour	2.86 ± 1.03^{a}	3.51 ± 1.07^{b}	3.42 ± 0.97^{b}

EM = entire males, SC = surgical castrates, G = gilts
[a,b] Values with different letters within rows are significantly different (min P< 0.05)

Table 5. Concentrations of androstenone and skatole in the adipose tissue

Item	EM	SC	G
Androstenone, µg/g	0.0079^{a}	0.0009^{b}	0.0018^{b}
Skatole, µg/g	0.3562^{a}	0.0350^{b}	0.0830^{b}

EM = entire males, SC = surgical castrates, G = gilts
[a,b] Values with different letters within rows are significantly different (P< 0.05)

Discussion

Pork quality between entire males and gilts in the presented study differed significantly only in pH_{45} in *semimembranosus* muscle. Significant difference between entire males and castrates was found only for EC_{24} in the same muscle. *Trefan et al. (2013)* reported difference between entire males and castrates for pH_{24} in *semimembranosus* muscle. *Nold et al. (1999)* found significant differences between boars, barrows and gilts. In the 100 kg final body weight boars had less WHC and lighter colour than gilts, whereas in the 110 kg final body weight no differences were observed in WHC and boars had a darker colour than gilts or barrows. Higher proportion of dark, firm and dry meat from boars compared to castrates or gilts is reported in the *EFSA review (2004)*. On the other hand, *D´Souza and Mullan (2003)* did not find any differences between entire males and surgical castrates in pH_u, meat colour L*, a*, b*, and drip loss.

Significantly lower evaluation of samples from entire males and castrates or gilts in our study was observed for pork odour, flavor, tenderness, overall perception as well as for fat odour and flavour. Large study on consumer acceptability of boar meat was performed in seven European countries *(Matthews et al., 2000)*. Consumers were dissatisfied 22 % of boar samples for flavour and 34 % for odour compared to 19 % and 28 %, respectively, for samples from gilts.

These differences between entire males and gilts were relatively small. A lot of studies found no differences in sensory quality between pork from entire males with low levels of androstenone and skatole related to pork from gilts and castrates. However, some studies reported lower acceptability of boar meat in spite of low levels of androstenone and skatole *(Bonneau et al., 1992; Babol et al., 1996; Siret et al., 1997; Font I Furnols et al., 2008)*. Significantly higher androstenone and skatole odour and flavour in meat of entire males than surgical castrates and females were reported by *Font I Furnols et al. (2009)*. Score for juiciness was also lower in entire males compared to other two types. No significant differences in odour and taste between entire males, surgical castrates, immunocastrates and gilts were reported in the study of *Jeong et al. (2008)*. However, pork from entire males was less tender than that of surgical castrates and females.

The results presented in this study suggest that sensory properties of meat and fat tissue were significantly worse rated in boars compared to gilts and surgical castrates in spite of lower concentration of androstenone in entire males. It seems that contribution of skatole to the boar taint was higher than that of androstenone.

Conclusion

Pork quality of entire male pigs was very similar to that of surgical castrates and gilts. Significantly lower values of boars were found only for electrical conductivity in *semimembranosus* muscle compared to castrates and for pH in the same muscle compared to gilts. However, large differences between boars and castrates or gilts were observed in sensory traits. Consumers rated better all these parameters except for juiciness in surgical castrates and gilts than in entire male pigs.

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Kvalitet mesa i senzorna prihvatljivost svinjskog mesa i masnog tkiva nekastriranih i kastriranih nerasta i nazimica

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Rezime

Cilj studije je bio da se procene parametri kvaliteta svinjskog mesa, kao i prihvatanje od strane potrošača mesa i masnog tkiva nekastriranih nerasta (EM) u odnosu na hirurški kastrirane (SC) i nazimice (G). Četrdeset dve svinje, po 14 za svaki pol, je slučajno izabrano za eksperiment. Svinje su melezi landras krmača i nerasta jorkišir x landras. Od sedam legala, 6 prasadi je izabrano (2 EM, 2 SC i 2 G). Nakon dostizanja prosečne težine pred klanje od 105 kg, svinje su zaklane u eksperimentalnoj klanici. Većina osobina kvalitetnog svinjskog mesa koje su bile predmet studije nisu pokazale nikakve značajne razlike među polovima. Međutim, tri parametra se statistički razlikuju između grupa svinja - pH vrednost merena 45 min nakon klanja u *semimembranosus* mišiću (MSM), električna provodljivost u istom mišića merena 24 časa post mortem i kalo. Nazimice su imala najveći pH_{45} MSM i nekastrirani nerasti najnižu (6,74 vs 6,48; $P<0,05$). Značajna razlika ($P<0.05$) u električnoj provodljivost je utvrđena između nekastriranih i kastriranih nerasta (3,27 vs 4,83 μS). Kalo je bio znatno niži kod kastriranih nerasta u odnosu na nazimice (2,30 vs 3,23%; $P<0.05$). Značajne razlike između nekastriranih nerasta i kastriranih ili nazimica, ili oboje, je zabeleženo u svim senzornim karakteristikama uzoraka mesa osim za sočnost, kao i za ukus i miris masnog tkiva.

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THE LEVEL OF POLYCYCLIC AROMATIC HYDROCARBONS IN FOOD - TRADITIONAL SMOKING OF MEAT AND MEAT PRODUCTS

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Invited paper

Abstract: New regulations regarding the maximum limits of polycyclic aromatic hydrocarbons (PAH) have caused a huge dispute among the processors and academics, causing confusion of the consumers. This work explains the history of the new regulations, their development and changes in recent years. It highlights the most important factors influencing PAH formation in traditionally smoked meat products and the methods to reduce their content in the final product. The study also summarizes and explains why the new regulations caused such a concern of the traditional processors and provides the insight about the problems associated with the new PAH regulations.

Key words: polycyclic aromatic hydrocarbons (PAH), traditionally smoked meat products

Introduction

Polycyclic aromatic hydrocarbons (PAHs) constitute a large class of organic compounds that are composed of two or more fused aromatic rings *(EFSA, 2008)*. When in 1866, Friedrich August Kekule von Stradonitz discovered the structure of benzene, it has become the basis for the description of the structure and properties of numerous and important group of organic compounds known as aromatic compounds, especially the polycyclic aromatic hydrocarbons (PAH).

Those include hundreds of compounds, occurring naturally in nature and in food products. They are a constant contaminants of the environment, which are created through incomplete combustion of hydrocarbons for example during forest fire or volcano eruption but also during waste incineration, asphalt production, cigarettes smoking or burning of wood, so naturally also during smoking *(Dutkiewicz, 1988)*. European Pollutant Release and Transfer Register (PRTR) on the basis data collected in 2011 among the EU countries, has estimated the benzo[a]pyrene (BaP) emission into the atmosphere on the level of 222 tons, and pointed that the highest emission sources are wood pulp and other fibrous products production industry and thermal power plants and other fuel combustion installations, which are responsible for almost 70% of PAH emission into the atmosphere *(Kiljanek et al., 2014)*. These PAH particles since the moment of emission are subjected to many chemical reactions and are deposited in the soil, on the surface of the plants and dissolving in water. Through those routes they are consumed by animals and humans. The animals research have shown that PAH can damage the cell genome and cause cancerogenesis. One of the features of some PAH is the presence of high electron density areas called “bay region”, which allows for creation adducts from the DNA of living organisms, which negatively affects the cell replication. The consequence is the long-term cancarogenesis. The most important PAH are: naphthalene, anthracene, phenanthrene, chrysene, naphthacene, pentacene, pyrene, BaP andhexacene *(Kubiak, 2013)*. *Sinha et al. (2005)* provide the evidence that dietary BaP intake plays a role in colorectal adenoma etiology. Those compounds can migrate through direct and indirect route into the food products,. The direct route consists of absorption of those compounds by plants from the soil and through the fall of PAH from the air with dust and rain - Table 1 and 2. The indirect route is usually associated with processing such as smoking, or drying of cereals in dryers powered by petroleum or through contamination of food additives with PAH.

The research performed by University of Warmia and Mazury in Poland *(Wieczorek and Wieczorek, 2011)* showed that 52.9% of daily consumption of PAHs comes from consumed grain products, 13.4% from meat and meat products, 8.7% from fats, 8.5% from milk and dairy products, 6.6% from vegetables, 4.2% from potatoes and 4% from fruits. In Tables 3 and 4 the contents of polycyclic aromatic hydrocarbons (PAHs) in food products are shown.

Table 1. Polycyclic aromatic hydrocarbons (PAHs) in the environment – soil. ***(Kuna, 2011)***

PAHs	Standard(µg/kg)	Scope (µg/kg)
Naftalen	100	5-87
Fenantren	100	22-763
Antracen	100	7-222
Fluoranten	100	26-1112
Benzo(a)antracen	100	7-153
Chryzen	100	16-386
Benzo(a)piren	30	11-159
Benzo(b)fluoranten	100	26-336
Benzo(k)fluoranten	100	8-202
Benzo(ghi)perylen	100	11-87
ΣPAH	1 000	115-3143

Table 2. Polycyclic aromatic hydrocarbons (PAHs) in the environment – rainwater. ***(Kuna, 2011)***

PAHs	Standard(µg/L)	Scope (µg/L)
Benzo(a)piren	0.1	0.01- 0.11
Benzo(b)fluoranten	0.03	0.01 – 0.23
Benzo(k)fluoranten	-	0 – 0.08
Benzo(ghi)perylen	0.002	0.01-0.14
Indeno(1,2,3cd)piren	-	0 – 0.08
0 - The limit of quantitation – 0,5ng/L		

Table 3. Contamination of food products B[a]P (µg/kg^{-1}) ***(Wilczyński, http://wsse.krakow.pl/strona2/index.php/publikacje)***

Food product	Concentrations B[a]P (µg kg^{-1})
Apples from the area clean	0.2 – 0.5
Apples from orchards in the area of urban	30 - 60
Margarine	1 – 36
Cabbage	25 – 40
Tea leaves	21
Bread	1
Lettuce	3 – 12
Toast	0.5
Dried flour	4
Natural roasted coffee and cereal coffee	1 - 13

Table 4. Concentrations (μg/kg^{-1} wet weight) of benzo(a)pyrene (B[a]P) and 4PAH (benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene) in different liquid and solid food samples

Food samples	B[a]P	4PAH (B[a]P + B[a]A + B[b]F + Ch)	References
Pomace oil	2.28-2.39	No data	*EFSA, 2008*
Coconut crude oil	40.6	263.6	*Węgrzyn et al., 2006*
Coconut refined oil	0.1	0.1	*Węgrzyn et al., 2006*
Rape-seed oil (cold pressed)	4.35	20.18	*Węgrzyn et al., 2006*
Universal oil (refined rape-seed)	0.24	1.49	*Węgrzyn et al., 2006*
Olive	0.69	4.12	*Węgrzyn et al., 2006*
Sunflower oil	0.27	0.93	*Węgrzyn et al., 2006*
Soya-bean oil	0.98	4.59	*Węgrzyn et al., 2006*
Smoked sausage	0.29	0.72	*Węgrzyn et al., 2006*
Smoked white cottage cheese	0.77	3.95	*Węgrzyn et al., 2006*
Smoked sprats	12.0	63.5	*Węgrzyn et al., 2006*
Milk powder	0.28	1.09	*Węgrzyn et al., 2006*
Bread and flours samples	0 – 16.5	No data	*Al-Rashdan et al., 2010*
Bread	0.16–19.1	No data	*Ciemniak and Chrąchol, 2008*
Cornflakes	0 – 0.12	No data	*Ciemniak and Chrąchol, 2008*
Oatmeal	Trace	No data	*Ciemniak and Chrąchol, 2008*
barley flakes	0.2 – 0.4	No data	*Ciemniak and Chrąchol, 2008*
Musli	trace – 1.8	No data	*Ciemniak and Chrąchol, 2008*
Crunchy	trace–1.07	No data	*Ciemniak and Chrąchol, 2008*
Hams - industrially smoked	0.37	No data	*Ciecierska and Obiedziński, 2007*
Cooked cured loins	0.30	No data	*Ciecierska and Obiedziński, 2007*
Raw cured loins	0.32	No data	*Ciecierska and Obiedziński, 2007*
Medium-ground sausages	0.89	No data	*Ciecierska and Obiedziński, 2007*
Ayam bakar	2.57–6.46	28.9 – 51.1	*Farhadian et al., 2010*
Ikan bakar	0 –1.23	9.36 - 15	*Farhadian et al., 2010*
Beef satay	7.34 - 12.5	81 - 132	*Farhadian et al., 2010*
Beef kebab	0 – 0.84	9.36 - 15	*Farhadian et al., 2010*
Chicken kebab	0 – 2.83	8.23 – 20.4	*Farhadian et al., 2010*
Cereals	0.2 – 0.4	No data	*EFSA, 2008*
Chocolate	0.3 – 0.32	No data	*EFSA, 2008*
Dried tea	8.37-8.39	No data	*EFSA, 2008*
Barbequed mea	1.92	No data	*EFSA, 2008*
Spices	3.50	No data	*EFSA, 2008*
Lard and dripping	0.4	No data	*European Commission, 2002*
Beer	0.04	No data	*European Commission, 2002*
Cereals bran	5.4	No data	*European Commission, 2002*
Sugar and sweets	0.15	No data	*European Commission, 2002*
Grapes	1.5	No data	*European Commission, 2002*
Grilled meat	157	No data	*European Commission, 2002*
Smoked herring	8.4	No data	*European Commission, 2002*
mussels from shellfish farm	5.9	No data	*European Commission, 2002*
Kale	4.2	No data	*European Commission, 2002*
Chicory	3.0	No data	*European Commission, 2002*

Oliver	11.3	No data	*European Commission, 2002*
Smoked pork chop	No data	1.18	*Mičulis et al., 2011*
Nuts ham-rolle	No data	1.82	*Mičulis et al., 2011*
Pork ham in net	No data	2.84	*Mičulis et al., 2011*
Breakfest ham	No data	8.22	*Mičulis et al., 2011*
Herring (smoked)	No data	20.2	*Mičulis et al., 2011*
Sprats in oil (smoked)	No data	18.14	*Mičulis et al., 2011*
Cod (smoked)	No data	7.05	*Mičulis et al., 2011*
Salmon (smoked)	No data	4.34	*Mičulis et al., 2011*
Hot smoked salmon fillet	18.73	53.92	*Food Standards Agency, 2012*
Smokies	12.9	39.62	*Food Standards Agency, 2012*
Roast smoked merguez sausage	2.14	6.4	*Food Standards Agency, 2012*
Smoked chicken breast	1.1	3.45	*Food Standards Agency, 2012*
Soup mix	1.26	3.0	*Food Standards Agency, 2012*
Muffle (t = 15 min)	1.2 – 2.1	No data	*Rey-Salgueiro et al., 2008*
Sausage - traditionally smoked	0.5 – 6.1	<34	own research data
Ham - traditionally smoked	0.5 – 5.6	<26	own research data
Bacon	0.5 – 2.6	<16	own research data
Kabanos	0.5 – 6.4	<34	own research data

The median dietary exposure across European countries was calculated both for mean and high dietary consumers and varied between 235 ng/day (3.9 ng/kg b.w. per day) and 389 ng/day (6.5 ng/kg b.w. per day) respectively for benzo[*a*]pyrene alone, 1168 ng/day (19.5 ng/kg b.w. per day) and 2068 ng/day (34.5 ng/kg b.w. per day) respectively for PAH4 . The two highest contributors to the dietary exposure were cereals and cereal products, and sea food and sea food products *(EFSA, 2008).*

Moreover, one burned cigarette equals 0.16 μg/kg of BaP, so smoking of the whole pack of cigarettes equals 3.2 μg/kg absorbed by the human organism. Smoking one pack of unfiltered cigarettes per day yields 0.7 μg/day benzo(a)pyrene exposure. Smoking a pack of filtered cigarettes per day yields 0.4 μg/day *(Sullivan and Krieger, 2001).*

The research on the toxicology of PAH has caused the Scientific Committee on Food of European Commission in 2002 to recognize 15 PAH compounds as genotoxic an cancerogenic for humans. In 2005 the Joint FAO/WHO Committee on Food Additives had 13 out of prevoiously mentioned 15 compounds as genotoxic and cancerogenic. The *Directive 208/2005* has introduced the maximal limit (ML) of BaP in food. For smoked meat, meat products, fish and fishery products the ML was established as 5.0 μg/kg and for unsmoked fish as 2.0 μg/kg. By answering the call of *EU recommendation no. 2005/108/WE* to further analyze the levles of 15 PAH in food products, 18 member countries have provided approximately 10 000 results of PAH analysis. Poland did not provide any result, despite the fact, that traditional smoking is the most common in this country. The elaboration of the acquired results was carried out by European Food Safety

Authority (*EFSA*), which concluded that it is possible to exacerbate the current PAH limits. In the *Directive 1881/2006*, the ML for BaP had not changed, and it had been concluded that since there is no threshold dose for genotoxic compounds, the levels of PAH should be as low as reasonably achievable (ALARA). This also included the compounds used in medicine. Due to *EFSA* recommendations, that the ML for PAH could be exacerbated, the following law regulations have been issued:

- *Commission Regulation 835/2011* of 19 August 2011, amending Regulation (EC) No 1881/2006 as regards maximum levels for polycyclic aromatic hydrocarbons in foodstuffs

- *Commission Regulation No 836/2011* of 19 August 2011amending Regulation (EC) No 333/2007 laying down the methods of sampling and analysis for the official control of the levels of lead, cadmium, mercury, inorganic tin, 3-MCPD and benzo(a)pyrene in foodstuffs

- *Commission Implementing Regulation (EU) No 1321/2013* of 10 December 2013establishing the Union list of authorised smoke flavouring primary products for use as such in or on foods and/or for the production of derived smoke flavourings

The Regulation 835/2011 has introduced from 1st September 2012 the new limits for BaP and the sum of 4 PAHs as 5.0 μg/kg and 30.0 μg/kg respectively for smoked meat and fish products (with exceptions), which have been further reduced from 1st September 2014 to the level of 2.0 μg/kg and 12.0 μg/kg respectively. The exacerbation of the limits seems well funded, but if the whole BaP and PAH dispute is supposed to protect the health of the consumers, then why:

- Cocoa seed and its byproduct (chocolate) have limits of 5.0 μg/kgBaP and 30.0 μg/kg of 4 PAH, even though children consume higher amounts of those products then of smoked meat products. The explanation provided in *Commission Regulation 835/2011* L215/5 (17), that it is mostly due to inappropriate process of drying of cocoa seed, does not seem justified.

- Smoked sprat will be subjected to old, higher limits of 5.0 μg/kg and 30.0 μg/kg meanwhile Polish carp will be subjected to exacerbated limits. Similarly, smoked clams will be subjected to mitigated limits (6 μg/kg of BaP and 35 μg/kg of 4 PAH).

- Coconut oil has mitigated limits of PAH (20.0 μg/kg of 4 PAH) while Polish rapeseed oil have exacerbated limit of 10 μg/kg of 4 PAH.

Moreover, one burned cigarette equals 0.16 μg/kg of BaP, so smoking of the whole pack of cigarettes equals 3.2 μg/kg absorbed by the human organism. So a pack of cigarettes provides more PAH than 1 kg of traditionally smoked sausage.

The actions performed by Polish Association of Traditionally Smoked Products Processors (ATSPP) and mutual cooperation in order to derogate the *Commission Regulation 1881/2006* of such countries as Ireland, Spain, Croatia, Cyprus, Latvia, Poland, Portugal, Romania, Slovakia, Finland, Sweden, and United Kingdom, resulted in issuing a new *Commission Regulation (EU) No 1327/2014* of 12 December 2014 amending Regulation (EC) No 1881/2006 as regards maximum levels of polycyclic aromatic hydrocarbons (PAHs) in traditionally smoked meat and meat products and traditionally smoked fish and fishery products.

Ireland, Spain, Croatia, Cyprus, Latvia, Poland, Portugal, Romania, Slovak Republic, Finland, Sweden and the United Kingdom may authorise the placing on their market the traditionally smoked meat and smoked meat products, smoked in their territory and intended for consumption in their territory with levels of PAHs higher than those set out in point 6.1.4. of the Annex *Commission Regulation (EU) No 1327/2014*, provided that those products comply with the maximum levels applicable before 1 September 2014, i.e. 5,0 μg/kg for benzo(a)pyrene and 30,0 μg/kg for the sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene. Simmilarly Ireland, Latvia, Romania, Finland, Sweden and the United Kingdom may authorise placing on their market the traditionally smoked fish and smoked fishery products, smoked in their territory and intended for consumption in their territory with levels of PAHs higher than those set out in point 6.1.5. of the Annex, provided that those smoked products comply with the maximum levels applicable before 1 September 2014, i.e. 5,0 μg/kg for benzo(a)pyrene and 30,0 μg/kg for the sum of benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene.

Those Member States are obliged to continue the monitoring of the presence of PAHs in traditionally smoked meat and smoked meat products and shall establish programmes to implement good smoking practices where possible, within the limits of what is economically feasible and what is possible without losing typical organoleptic characteristics of those products.

Within 3 years from the application of the Regulation, the situation will be re-assessed, on the basis of all available information, in view of determining a list of smoked meat and smoked meat products for which the derogation for local production and consumption shall be continued without a time limit.

On this basis, the Polish Ministry of Agriculture and Rural Development issued *Regulation No 1845* of 15 December 2014 regarding veterinary requirements during the production of smoked meat products in relation to the

maximum contamination limits of Polycyclic Aromatic Carbohydrates (PAH). Until the 31st of August 2017 it is allowed to produce and introduce into the market, only on the territory of Poland, smoked meat product, produced only in Poland, without the addition of smoke aroma in which the critical limit of contamination for: BaP is not more than 5.0 μg/kg and the sum of 4 PAH (benzo(a)pyrene, benz(a)anthracene, benzo(b)fluoranthene and chrysene) does not exceed 30.0 μg/kg. Traditionally smoked meat products, to which the exception is applicable are marked with special square-shaped veterinary sign.

This regulation is a compromise, which gives time for a thorough monitoring of the PAH level contamination within the traditionally smoked meat products.

Smoking is a process of saturating with smoke the food products which have been cured or salted and dried. The saturation with smoke ingredients may take place by the means of diffusion from the smoke-air mixture, through application of liquid layer on the surface of a product or through addition of liquid smoke into the fragmented product. The synergic effect of smoke, temperature, sodium chloride and other chemical compounds provides the smoked product with specific favorable aroma and taste. Drying, especially surface drying, increasesthe nutritional value and gives antiseptic properties to the food product, increasing its shelf-life. Dehydration of the product reduces the water activity thus preventing the bacterial and fungal growth and inactivates the enzymes. The protein denaturation upon exposure to the high temperature makes the product more favorable for consumption. The following main techniques of smoking are currently used:

1. Smoking in air and smoke environment
    - Conventional (regular) – in neutral air and smoke environment
    - Electrostatic – in high voltage electric field
2. Smoking in smoke solutions (liquid smoke) *(Kołakowski, 2012)*

Smoking in air and smoke environment includes also the traditional smoking, which takes place in traditional smoking chambers, where the smoke and heat is generated through burning of thick pieces of deciduous tree wood, with proper humidity. The furnace is located inside the smoking chamber, and above it, in certain distance, the product is hanging on the smoking sticks. Smoking in the air and smoke environment can be further divided into cold smoking (16-22^{o}C), warm smoking (22-40^{o}C) hot smoking (40-90^{o}C) and hot smoking connected with baking. In industrial conditions, the smoke is generated through incandescence, friction, steaming or fluidal pyrolysis of sawdust or smoking chips *(Pisula and Pospiech, 2011).* Smoke consists of few hundreds of compounds which can

favorably influence the quality of smoked product, be neutral for the consumer or can be potentially hazardous for the consumers health. Formaldehyde, which comes from smoking, reacts with proteins, reducing their digestibility, in the oversmoked products. During smoking, phenols, play a crucial role in the creation of specific sensory properties of the smoked product. Moreover they exhibit strong antioxidant properties.

What is traditional smoking? We propose the following definition: Traditional smoking is a process, performed in accordance with artistry and knowledge of local producers, which includes drying, cold, warm and hot smoking and hot smoking and baking, to receive pale, dark, brown, cherry etc. color, depending on centuries old local tradition, conducted in traditional smoke chambers, in which the smoke and temperature is generated from burning of thick deciduous tree wood, with proper humidity in a fireplace located directly inside the chamber and above which in a certain distance the smoked and processed product is located, hanging on the smoking sticks.

The preliminary monitoring performed by Veterinary Inspection in 2014 showed, that 38% of studied smoked meat samples exceeds the nee limits (65 out of 170, from which 51 were sausages). On the basis of our research and research performed by the producers, we can report that the final effect of the smoking, thus the amount of PAH in the final product depends on number of factors, from which the most important are:

- Raw material – research suggest that raw meat contains from 0.0 – 0.9 µg of BaP/kg. This can be the effect of contamination of soil, water and air and accumulation of PAH in the plants which constitute of animal fodder *(Kuna, 2011).*

- Type of raw material and fat content – poultry meat products contained lower amounts of BaP (0.9 µg/kg) than in pork meat products. *Waszkiewicz-Robak et al. (2014)* concluded that the overall amount of PAH which are formed during smoking of product increases when the raw material contains more fat. Moreover they reported that the amount of so called heavy PAH, which are formed during smoking, depends on the fatty acids composition of the raw material and is positively correlated with higher unsaturated fatty acids content. The addition of oils into the animal fodder (such as fish or flaxseed oil) influenced the changes in fatty acids composition, which in turned increased the PAH formation during smoking, especially the heavy PAH, such as BaP. This could also be caused by the contamination of vegetable oils with PAH *(Ciecierska and Obiedziński, 2006).* There is a necessity of determination of the PAH level in individual food products, since in the EU reports, which were published in 2004 and 2008, there was a

lack of the PAH level in the smoked food products and in the environmental samples from Poland. The lowest PAH contamination was detected in poultry products which indicates the great importance of the effect of raw material that is smoked. Poultry products are usually subjected to mild smoking, applying different parameters than it is in the case of other meats *(Kubiak, 2012).*

- Type of meat product – type of sausage, its thickness and composition have influenced both the amount of BaP and the total PAH. The most problematic sausage is the kabanos sausage – Polish long, thin dry sausage. Large surface, low mass and thin profile cause high fluctuations in PAH content: the BaP content ranges from 0.9 µg/kg (poultry kabanos) till 9.1 µg/kg while the sum of 4 PAH reaches 45.0 µg/kg. Only short smoking followed by baking allowed to reduce the amount of those compounds

- Spices and food additives – the quality of spices, drying method, the quality of added soy protein or fiber affects the quality of the final product. According to *Kubiak (2013)* the more common use of smoking additives to improve the quality and sensory properties of the product has become a significant source of PAH in meat products.

- Type of casing – natural casings are not a barrier for the PAH during smoking. Moreover, during analysis, the sausage is grinded together with the casing, which is treated as an edible part of the sausage. The use of collagen protein casings in the same sausage significantly reduces the amount of PAH inside the product. Moreover the use of collagen protein casings with thick overprint reduced the BaP in the sausage to 0.9 µg/kg and the sum of 4 PAH to 8 µg/kg. During the analysis, the sausage is grinded without the casing and the overprint acts as a barrier for the PAH. In order to reduce the PAH content in the meat products, processors should use the artificial casings which stop the PAH on the surface and decrease their migration into the inner layers of the product. The cellulose casing in much higher degree stops the smoke compounds from migration into the product. However the traditional products in artificial casing might not be accepted by the consumer.

- Type of heat treatment – this parameter is connected with the smoking temperature. Fast smoking, so called “smoke peck” and then baking in 85^{o}C reduces the amount of PAH, so that the product does not exceed the new limits. This method is recommended especially to smoke meat products with high fat content such as beacon or sausage.

- Smoking temperature –generally, the higher temperature of smoking, the more PAH in the final product. The content of BaP during cold and warm smoking was reported to decrease (approx. 1.7 µg/kg). Pale smoking however resulted in a loss of attractiveness for the consumers. Consumers in Lesser Poland and Subcarpathia are used to meat products with dark, brown or cherry smoking color, depending on the local tradition, while the pale smoked products are treated as not fully smoked or unfinished.

- Drying degree – our research performed on smoked meat products and sausages which surface was not dried before smoking showed higher accumulation of PAHs (up to 10 µg/kg of BaP and 85 µg/kg of sum of four PAHs) while when the same products with previously dried surface in the temperature of 80^{o}C for around 90 minutes contained much lower PAH content (1.8 µg/kg of BaP and 21 µg/kg of the sum of four PAHs). The wet surface of the casing accumulates more smoke ingredients, especially of the so called thick smoke.

- Type and construction of the smokehouse –the subject of new smokehouse construction, which includes moving the fireplace out of the smoking chamber and using alternative smoking methods, often recommended by various experts, is the attempt to destroy the local tradition and to eliminate the traditionally smoked products in the area. This is in direct contrast to the idea of so called “Small Homelands” and preservation of local tradition and heritage propagated by the European Union. Those ideas suggest preservation of traditionally smoked products. Moving the fireplace out of the smoking chamber is the favoritism of cold and warm smoking. Suggesting that meat products from Lesser Poland and Subcarpathia are dripping with tar and burned fat shows ill will. Almost every smoker and meat processor will not allow the drip of fat from the product, because such product would lose its succulence and taste – the basic sensory attributes, and will be unacceptable for the consumer. Intense drying of the product in 60-80^{o}C and then smoking and baking in 75-80^{o}C leads to “closing of the product” and will prevent the fat loss.

- Smoking wood – the type, hardness and humidity. The best smoking effect is achieved on the wood from fruit trees, which is acquired from chopping down old trees or yearly undercutting of the fruiting trees, especially plum-tree, cherry-tree, gean-tree, apricot-tree or apple-tree, because the wood of those trees is rich in hemicellulose. The highest hemicellulose content is present in apricot-tree (approx. 40%), apple-tree (37%) and pear-tree (32%), while the hemicellulose content of non-horticultural trees is usually between

25-30%. Walnut-tree should be avoided due to intense bitter taste of the final product. Wood type also influences the color of the final product. Smoke from beech, maple ash-tree or linden gives the product a specific golden-brown or golden-yellow color, while the smoke from pear-tree or apple-tree gives red to dark brown color. Oak wood smoke provides dark-gold to brown color. In Poland, the trees which are most commonly used for smoking is alder, which gives a specific color – from lemon to brown, depending on the smoke concentration. Moreover, when alder humidity is not appropriate, it has the tendency to tar the product. Alder is a cheap and efficient wood, which also contains low levels of tannins (3-5%), which results in low bitterness of the final product. The recommendation of using the hard wood for smoking has its grounds, because during pyrolysis of hard wood the formation of BaP is reduced 1.5-4.5 times when compared to soft woods (pine, fir) *(Kowalski and Pyrcz, 2006).* Hard wood, with tight structure burns more slowly, and the volatiles formed during such process are less prone to oxidation into carbon dioxide, as it happens when burning the soft wood. The wood for smoking should have hardness of >40 MPa and volumetric mass density of 0.500 g/cm^3. Such parameters are met by various deciduous trees such as ash-tree, beech, hornbeam, maple, oak, elm, acacia or pear-tree because with increase in wood hardness there is also an increase in efficiency of acquired smoke compounds. Oak tree seems controversial, due to high tannin content of 4-10% in the wood, 5-17% in the bark and 20-45% in the leafs *(Surmiński, 2000).* Not every consumer, especially in Lesser Poland, tolerates the bitter, "oakish" taste of meat products. The wood used in smoking should be dry, which means its humidity should range between 15-20%. The use of wet (above 30% of humidity) and dry (less then 10% of humidity) should be avoided. Also it should be prohibited to use moldy wood, wood with putrefaction etc. Wood from dismantling of old house also should be avoided.

- Smoke generation techniques – the traditional smoking is dependent on the atmospheric conditions. Humidity, temperature and movement of air outside of the smokehouse will affect the final result of the process, because it affects the temperature of wood combustion. The use of modern industrial smoking chambers with smoke generators eliminates the problem of ensuring the proper combustion temperature, meanwhile in traditional smoking this process has to be maintained by the skills and experience of the smoker.

- Wood burning temperature – the detectable amounts of PAH are reported in the temperature above 400^{o}C however BaP appears clearly only after

reaching 500^{o}C *(McGrath, 2003)* with the peak of formation within 800-900^{o}C. Product smoked in the smoke generated in high temperature could be hazardous for consumers health, since it contains higher amounts of PAH, but also the product is less aromatic. The maximum temperature of wood combustion should not exceed 425-450^{o}C *(Kowalski and Pyrcz, 2006)*. Furthermore, the smoke acquired with small air inlet contains lower BaP content than the smoke generated with full air inlet. Due to this it is important to install air flow regulating latches into the smokehouse. The speed of volatiles evacuation from the combustion area also influences the amount of PAH in the final product. The smoke generated in low combustion (pyrolysis) temperature contains higher amount of acids and lower amount of phenolic compounds. Meat products smoked in such way are characterized by acidic aroma and loose, less preferable texture of meat.

- The sample collection method – the sample collected for the analysis should be uniform and representative. Samples of meat products which are closer to smoking stick (sausage hanging on the stick), contained lower PAH content then the sample of the sausage collected further from the stick. This is particularly important in sausages, because the fat settles on the lowest parts of the product. The research shows that the PAHs content in the inner part of a product is statistically significantly lower comparing to the outer part of the same product. The PAHs penetration is not so intensive, so peeling the casing off decreases significantly the PAHs content in the product. *(Ciecierska and Obiedziński, 2012).* The methods and techniques of sample collection for PAH analysis are regulated by the *Commission Regulation No 836/2011*.

- Analysis methodology – accredited laboratories which analyze the PAH content in food products of animal origins use different analytical methods: gas chromatography with mass spectrophotometry or HPLC. Despite the validation, those methods are incomparable (*Wenzl et al., 2006*).

- In the case of traditional smokehouses, based on natural air flow or convection, the critical point in reduction of PAH in the final product is the experience and skills of the smoker, who has to control the conditions of combustion. Experts' advices are valuable, but should be based on their own experience. Traditional smokehouse and traditional smoking is not the same, as modern computerized industrial smoking chamber.

Conclusion

Polycyclic aromatic hydrocarbons (PAHs) are a group of over 100 different chemicals. They may be dangerous for humans and animals that is why the regulations limiting their content in food are implemented. The most important PAHs are: naphthalene, anthracene, phenanthrene, chrysene, naphthacene, pentacene, pyrene, BaP andhexacene. They are constant contaminants of the environment, which are created through incomplete combustion of hydrocarbons for example during forest fire or volcano eruption but also during waste incineration, asphalt production, cigarettes smoking or burning of wood, so naturally also during smoking. Into the food products, those compounds can migrate through absorption of those compounds by plants from the soil, air with dust and rain, with processing such as smoking, or drying of cereals in dryers powered by petroleum or through contamination of food additives with PAH.

Meat and meat products smoked traditionally may contain more PAHs and the amount depends on many factors. Polycyclic aromatic hydrocarbons (PAHs) are natural antioxidants , so reducing their content in meat products may cause the decrease of the products' shelf-life. Moreover PAHs influence the flavor of smoked products and lowering the PAHs content, by changing the smoking technology, may lead to creating a new product, which may not be accepted by the consumer.

Nivo policikličnih aromatičnih ugljovodonika u hrani - Tradicionalno dimljenje mesa i mesnih prerađevina

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Rezime

Novi propisi u vezi maksimalne granice policikličnih aromatičnih ugljovodonika - PAU (PAH) izazvali su veliki spor između prerađivača i naučnika, izazivajući konfuziju potrošača. Ovaj rad objašnjava istoriju novih propisa, njihov razvoj i promene u poslednjih nekoliko godina. On naglašava najvažnije faktore koji utiču na formiranje PAU (PAH) u tradicionalno suvomesnatim proizvodima i metoda za smanjenje njihovog sadržaja u finalnom proizvodu. Studija takođe rezimira i objašnjava zašto su novi propisi izazvali takvu zabrinutost tradicionalnih prerađivača i pruža uvid o problemima u vezi sa novim PAU (PAH) propisima.

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THE EFFECTS OF FEEDING PIGS WITH CONJUGATED LINOLEIC ACID ON MEAT QUALITY

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Abstract: Conjugated linoleic acid (CLA) is a mixture of the geometric isomers of linoleic acid. Studies have found that feeding animals with the addition of CLA has various positive effects, such as improved feed conversion, reduction of the amount of fat tissue and improvement of the quality of the resulting meat. The reason for the great interest in this compound is also its potential health effects on humans, such as anticancer effect, enhancing the immune system, reducing the occurrence of cardiovascular diseases and the like. Feeding pigs with the addition of CLA is gaining importance in recent years, because it affects the reduction of the thickness and share of subcutaneous fat tissue and the increase of lean meat/meat yield of the carcass without negative impacts on the quality of the resulting meat. Increased content of intramuscular fat, i.e. marbling in meat and fatty-acid changes in the composition of meat have been determined. Increasing the content of saturated fatty acids in the fat and muscle tissue improves technological characteristics and oxidative stability of meat. In addition, due to the incorporation of CLA isomers in the tissues of animals and its health benefits, this type of diet can be a method for obtaining the pork with the features "of functional foods." However, all these positive effects have not been confirmed in each of the experiments focusing on feeding pigs with CLA, because there are many factors that affect its efficiency.

Key words: conjugated linoleic acid, pig, meat quality

Introduction

The composition of the slaughter carcasses is influenced by the breed, gender, diet and housing conditions (*Wagner et al., 1999*), and these factors have an effect on the composition and quality of the resulting meat. Besides them, the quality of meat is also affected by the conditions in animal transport, time spent in

the depot sacrifices, the stunning and primary processing, and cooling conditions and storage *(Stanišić et al., 2012b)*.

In recent years, the efforts of science and practice were focused on the reduction of body fat in carcasses of fattening animals, especially pigs (*Stanišić et al., 2011a)*. As a result, first of all of the improvement of the genetic basis of modern high-quality pig breeds and their crosses, as well as progress in the field of animal nutrition, the thickness of fatty tissue at the back part is reduced to below 10 mm, and the content of muscle tissue in carcass often exceeds 60%. Pigs today are bred/selected to have an efficient feed conversion and to better convert the food into muscle tissue *(Schinckel et al., 2001)*. However, this increase of meatiness had a negative impact on the quality of the resulting meat. There was a negative correlation between the increased share of muscle tissue and some sensory and technological parameters of meat quality, especially subcutaneous fat, as one of the main ingredients for the production of quality meat products *(Stanišić et al., 2011b, 2012a)*.

In practice, there are many ways and means which may be used to balance the growth of muscle and fat tissue in the body of animals and produce meat of desired quality. Latest developments in biotechnology have enabled scientists to influence the growth and distribution of certain tissues of animals through modification of their metabolism. Metabolic modifiers, such as β-agonists, anabolic steroids, somatotropin, vitamin E and trace elements and lipids with metabolic effect, such as conjugated linoleic acid, are used in order to improve performance and meatiness of fattening animals.

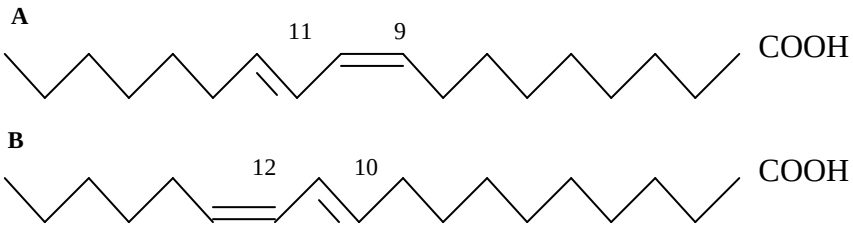
Conjugated linoleic acid (CLA) was first identified in 1935 after an analysis of cow's milk fat using UV spectrophotometer (*Booth et al., 1935*). However, the nutritional potential of CLA was recognized only in 1978, when Dr. Michael Pariza and his associates isolated a substance from roasted beef which showed mutagenic effects (*Pariza et al., 1979*). Later research has shown that this "mutagen" has anti-carcinogenic effects and that it was in fact cisΔ^9, transΔ^{11} conjugated linoleic acid derivative *(Pariza and Hargaves, 1985; Ha et al., 1987)*. Since then, a number of experiments was performed to study its functional and structural aspects.

CLA is found naturally in milk and fat tissue of ruminants, and formed in the rumen through bacterial biohydrogenation from linoleic acid, however, only a small amount of CLA (about 0.6 mg/g fat) is in pork meat (*Chin et al., 1992*) . The only way to increase its share in the pork is adding chemically synthesized CLA in the diet (*Dugan et al., 1997*). Feeding pigs diet with the addition of CLA is gaining importance in recent years because it improves the characteristics of the carcass *(Stanišić et al., 2013)* and quality of pig meat, and at the same time, it is a way of obtaining meat and meat products enriched with CLA (*Lauridsen et al., 2005*).

Synthesis and mechanism of action of CLA

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

CLA is naturally occurring through the bacterial biohydrogenation of linoleic acid (18: 2 n-6) in the rumen of ruminants by means of several kinds of microorganisms (*Griinari et al., 2000*). Consequently, food derived from ruminants (meat and milk) is the main source of CLA in the human diet (*Chin et al., 1992*). Although the food contains a large number of isomers of CLA, the most common are cisΔ^{9}, transΔ^{11} (cis-9, trans-11) and transΔ^{10}, cisΔ^{12} (trans-10, cis-12) (Picture 1).



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

There are several mechanisms of action of CLA, but the majority are still not fully explained, and one reason for this, as stated by *Yang et al. (2000),* is that the CLA oxidizes faster than linoleic acid, which would mean that the conjugated double bond is more susceptible to oxidation than unconjugated. Finding that CLA is more susceptible to oxidation, i.e. that it can be oxidized prior to its utilization, suggests that its effects can be reduced, and this may be one of the reasons for variations in the results of its effects between different experiments.

Several researchers found that CLA affects the increase in energy consumption, because increased oxygen consumption of animals fed CLA was established in the trials *(West et al., 2000),* which represents one of the mechanisms that influence the reduction of fat tissue in the body of animals. Another mechanism for reducing the amount of fat tissue under the influence of CLA is the

impact on reduction of the size and/or number of fat cells in the fat *(Pastoreli et al., 2005)*. This can be achieved by inhibition of the enzyme lipoprotein lipase in adipocytes, inhibiting the activity of the enzyme of the sterol-CoA desaturase. Since the lipoprotein lipase is a key enzyme which regulates the deposition of fat in the body, its inhibition will induce the reduction of adipogenesis, which was confirmed for the effect of the trans-10, cis-12 isomers of CLA, but not for cis-9, trans-11 isomer *(Park et al., 1997)*. Sterol-CoA desaturase is an enzyme which regulates the conversion of saturated to mono-unsaturated fatty acids, which are the main constituents of the subcutaneous fat tissue, so its inhibition can influence the reduction of the quantity of fat tissue *(Park et al., 2000; Lin et al., 2004)*. As a consequence of the above, the addition of CLA in the diet of pigs can decrease the accessibility of fatty acids for the synthesis of triglycerides and reduce the amount of fat, as found by several authors *(Pastoreli et al., 2005)*.

Feeding animals with CLA affects the change of fatty acid composition of fat in muscle and adipose tissue. On the basis of investigations that the CLA reduces the content of monounsaturated fatty acids (MUFA) in animal fats, *Evans et al. (2000)* have found that the trans-10, cis-12 CLA isomer is responsible for this decrease. However, the mechanism of action of CLA on reducing the MUFA and increasing the saturated fatty acids (SFA) has not been fully explained. *Lee et al. (1994)* assume that the effect of CLA on saturation of fatty acids manifests through the inhibition of the enzyme activity, Δ-9 sterol-CoA desaturase. This theory was confirmed by *Pariza et al. (2000).*

Some of the effects of feeding pigs with CLA, which have been documented in scientific publications, are: improved feed conversion, reducing the thickness of subcutaneous fat, increased marbling of meat, increased firmness of body fat, improved meat color, change in the fatty acid composition of meat and reduced susceptibility to oxidation. However, all these positive effects are not confirmed in every experiment which included feeding pigs with CLA, because there are many factors that affect its efficiency.

The effect of CLA on quality of pig meat

CLA is thought to positively influence the quality of pork. Studies have found that the thickness of subcutaneous back fat decreases on average by about 1.2 mm, but the amount of intramscular fat increases by about 7%, in pigs fed CLA *(Dunshee et al., 2005)*. This increase of the content of intramuscular fat or marbling of the meat would mean that the feeding pisg with CLA improves the sensory quality of the meat. However, most authors did not identify significant effect of CLA on sensory scores of tenderness, juiciness and flavor of roasted meat *(Thiel et al., 1998; Dugan et al., 1999; Thiel-Cooper et al., 2001; Wiegand et al.,*

2002; Larsen et al., 2009), which are positively correlated with the content of intramuscular fat.

In several studies positive effects of feeding animals with CLA on the parameters of technological quality of meat are revealed. In this regard, water holding capacity of meat from pigs fed CLA is either enhanced *(O'Quinn et al., 1998; Joo et al., 2002; Szymczyk, 2005)* or remains unchanged *(Thiel-Cooper et al., 1999; Wiegand et al., 1999).*

Influence of feeding pigs with CLA on instrumental meat color differs between studies. *Wiegand et al. (2001)* report a significant increase in L* and a* and decrease in b* values of meat color from pigs fed CLA. On the other hand, some researchers did not determine the impact of CLA on the change of the meat color *(O'Quinn et al., 1998; Dugan et al., 1999; Martin et al., 2007; Barnes et al., 2012).*

As regards the pH value, *Wiegand et al. (2001)* report that pigs fed with the addition of CLA had a lower pH value of the meat, as measured 3 hours post-mortem, while the pH value 24 hours after slaughter was not different compared to the control. Other researchers suggest that CLA has no influence on the final pH value *(Thiel-Cooper et al., 1999; Dugan et al., 1999; Wiegand et al., 2001; Eggert et al., 2001).*

Feeding pigs with CLA affects a significant change in the fatty acid composition of fat and muscle tissue, which leads to the incorporation of CLA isomers in subcutaneous fat tissue and to a lesser extent in intramuscular fat *(Eggert et al., 2001; Ramsay et al., 2001; Wiegand et al., 2001; Joo et al., 2002).* The amount of isomers that is absorbed on this occasion and incorporated into the tissues depends on their concentration in food *(Ostrowska et al., 2003)*. The effect of dietary CLA on fatty acid composition of fat and muscle tissue is largely dependent on the type of fats/oils that are used as food and that are replaced by CLA, because some differences in the composition of fatty acids derive from the differences in the composition of the dietary fat/oil used in control and experimental groups. In this regard, *Dunshee et al. (2005)* suggest that in the interpretation of the effects of dietary CLA on fat composition, the fatty acid composition of the food used in fattening should be taken into account. Despite this fact, there are certain changes in the fatty acid composition of pork, attributed to the functioning of the CLA. For example, *Ostrowska et al. (2003)* found that the proportion of palmitic (16:0) and palmitoleic (16:1) acids, in the intramuscular and subcutaneous fat tissue increase linearly with an increase in the level of CLA in food. Some researchers indicate a considerable reduction in the shares of oleic (18:1), linoleic (18:2), linolenic (18:3), and arachidonic (20:4) acids in pigs fed CLA *(Chin et al., 1994; Ramsay et al., 2001; Joo et al., 2002; Sun et al., 2004)*. As a result of the aforesaid changes in the fatty acid composition, there is an increase in the share of SFA, and reduction in the share of unsaturated fatty acids *(Joo et al.,*

2002; Wiegand et al., 2002; Tishendorf et al., 2002; Szymczyk, 2005; Lauridsen et al., 2005), also an increase in the iodine number/value of the fat (*Eggert et al., 2001*), and thus increase of the hardness of fat tissue and improvement of its technological characteristics *(Eggert et al., 2001; Thiel-Cooper et al., 2001).*

Table 1. Summary of published reports on the effect of CLA on meat quality of pigs

Reference	% CLA	Breed[1]	Sex	Weight range (kg)	IM[2]	Fatty acid		Colour (CIE)[5]	WBC[6]	pH
						SFA[3]	UFA[4]			
Dugan et al. (1997)	2	L x VJ	♀ i ♂	62-106	↑	-	-	nc	nc	nc
Ostrowska et al. (1999)	0,7-5,5	VJ x L	♀	57-107	-	-	-	-	-	-
Thiel-Cooper et al. (2001)	0,07-0,6	(JxL)x(DxH)	♂	26-114	↓	↑	↓	-	-	-
Ramsay et al. (2001)	0,17-1,34	J x L	♀ i ♂	20-55	-	↑	↓	-	-	-
Wiegand et al. (2002)	0,75	(JxL)x(DxH)	♂	28-115	↑	↑	-	↑ b*	-	-
Joo et al. (2002)	1-5	L x VJ x D	♀	64-99	↑	↑	↓	-	↑	-
Szymczyk (2005)	0,1-0,6	L x VJ	♀ i ♂	61-108	↓	↑	↓	↑ L*	↑	nc
Lauridsen et al. (2005)	0,3	L x J x D	♂	40-130	nc	↑	↓	-	nc	nc
Martin et al. (2007)	0,6-1,2	VJ	♀	70-107	↑	↑	↓	nc	-	nc
Barnes et al. (2012)	0,6	PIC 380	♂	53-103	-	↑	↓	nc	-	-

nc – No change; ↑ – Increase; ↓ – Decrease.
[1] LW– Large White; Y – Yorkshire; L – Landrace; D – Duroc; H – Hampshire;
[2] IM – Intramuscular fat; [3] SFA – Saturated fatty acids; [4] UFA – Unsaturated fatty acids;
[5] CIE – CIEL*a*b* system *(CIE, 1976)*; [6] WBC – Water binding capacity.

In examining the impact of CLA in the diet of pigs on the quality of loin *M. longisiimus dorsi* muscle, *Joo et al. (2002)* report that the concentration of TBARS (tiobarbituric acid reactive supstances) is lower in pigs fed CLA after 7 days of storage at 4°C. Those authors suggest that the difference in oxidative stability of fats may be the result of increasing the share of total saturated fatty acids and decreasing the share of polyunsaturated fatty acids. *Ha et al. (1990)* have found that the CLA acts as an antioxidant, of larger capacity than α-tocopherol, and compared it with the β-hydroxytoluene (BHT). However, recent research has put into question the antioxidant effect of CLA, because of its susceptibility to auto-oxidation *(Yang et al., 2000).*

Table 1 shows the summarized published data on the effects of feeding pigs with CLA on the quality of the resulting meat.

Conclusion

Conjugated linoleic acid (CLA) is a mixture of geometric isomers of cisΔ^9, cisΔ^{12} – octadecadienoic (linoleic acid - 18:2 n-6) acid, for which many positive effects were identified, such as anticancer effect, improved feed conversion and reduction of the amount of fat and increase of the share of muscle tissue in the body of animals.

Feeding pigs with the addition of CLA is gaining importance in recent years, because it affects the reduction of thickness and the share of subcutaneous fat and increases the lean meat/meat yield in carcass without negative impacts on the quality of the resulting meat. Increasing the content of saturated fatty acids in the fat and muscle tissue improves technological characteristics and oxidative stability of meat. In addition, due to the incorporation of CLA isomers in the tissues of animals and its health benefits, this type of nutrition can be a method for obtaining the pork with the features "of functional foods." However, all these positive effects have not been confirmed in every experiment that included feeding pigs with CLA, because there are many factors that affect its efficiency.

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Uticaj ishrane svinja sa konjugovanom linolnom kiselinom na kvalitet mesa

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Rezime

Konjugovana linolna kiselina (CLA) predstavlja smešu geometrijskih izomera linolne kiseline. Istraživanja su utvrdila da ishrana životinja sa dodatkom CLA ima razne pozitivne efekte, kao što su poboljšana konverzija hrane, smanjenje količine masnog tkiva i poboljšanje kvaliteta dobijenog mesa. Razlog velikog interesovanja za ovo jedinjenje su i njeni potencijalni zdravstveni efekti na ljude, kao što su antikancerogeno dejstvo, poboljšanje rada imunog sistema, smanjenje nastanka kardiovaskularnih bolesti i sl.

Ishrana svinja sa dodatkom CLA dobija sve veći značaj poslednjih godina, jer utiče na smanjenje debljine i udela potkožnog masnog tkiva i na povećanje mesnatosti trupa, bez negativnih uticaja na kvalitet dobijenog mesa. Utvrđeno je povećanje sadržaja intramuskularne masti, tj. marmoriranosti mesa i promena masno-kiselinskog sastava mesa. Povećanje sadržaja zasićenih masnih kiselina u masnom i mišićnom tkivu poboljšava tehnološke karakteristike i oksidativnu stabilnost mesa. Dodatno, zbog inkorporacije CLA izomera u tkiva životinja i njenih zdravstvenih koristi, ovakva vrsta ishrane može biti i metod za dobijanje svinjskog mesa sa osobinama "funkcionlne hrane". Međutim, svi ovi pozitivni efekti nisu potvrđeni u svakom ogledu ishrane svinja sa CLA, jer postoje više faktora koji utiču na njenu efikasnost.

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COMPARISON OF THE FATTY ACID COMPOSITION OF FIVE DIFFERENT FAT TISSUES IN SWEDISH LANDRACE PIG

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Abstract: Fat is one of the most important edible products derived from slaughtered pigs and its fatty acid composition depends, among others, on the location in the carcass. The main objective of the present study was to compare the fatty acid composition of the five different fat tissues (taken from the lard, subcutaneous fat from back area, intramuscular fat from *M. Longissimus dorsi*, intermuscular fat from round and subcutaneous fat from belly region) in Swedish Landrace pig breed. A significantly higher share of total polyunsaturated fatty acid content was found in intermuscular fat (especially higher share of linoleic (18:2 n-6) and γ-linolenic (18:3 n-3) acid), compared to other fat depots ($p<0.05$). Total saturated fatty acid content was highest in lard fat, mainly due to a higher share of stearic acid (18:0). The highest share of total n-6 fatty acids was found in intermuscular fat, while intramuscular fat had the highest n-6/n-3 ratio (which is inconsistent with the nutritional recommendable values). Subcutaneous fat from back and belly region had similar content of major fatty acids. The obtained data confirm that the various pig adipose tissues are different in their fatty acid composition.

Keywords: pig fat tissue, fatty acid, Swedish Landrace pig breed

Introduction

The main parameters for the evaluation of pig carcasses are its weight, yield of warm and cold carcass, conformation and the ratio of muscle and fat tissue. The amount of muscle tissue is certainly the most important factor, but the distribution and quantity of fat tissue plays an important role in the value of the carcass, since excessive proportion of body fat (above the optimum) can have a very negative economic impact. Excess removable fat (intermuscular or subcutaneous), which is

removed after cutting and processing, represent an economic loss for producers and processors *(Harper et al., 2001)*. As a result, particularly of the improvement of the genetic basis as well as progress in the field of animal nutrition, the fat tissue content in pigs is reduced*(Stanišić et al., 2011; 2013)*, however, with a negative impact on its physical and chemical characteristics *(Allen and Foegeding, 1981)*.

Fat is one of the most important edible products derived from slaughtered pigs and one of the main ingredients for the production of quality meat products *(Delgado et al., 2002; Stanišić et al., 2012)* and its too low content in the carcass may have a negative economic effect. There are different fat depots in pig carcass: visceral, subcutaneous, intermuscular (between muscles), or intramuscular (within muscle). Intermuscular fat tissue, and smaller portion of subcutaneous fat tissue is sold together with meat or used for the processing of the meat of various categories. Fat tissue can be used as an ingredient for making various meat products (sausages, canned and smoked meat products), for the manufacture of emulsions in sausage production, or processed into edible animal fats.

The fatty acids composition of the pig body fat are influenced by factors such as feeding *(Leszczynski et al., 1992; Scheeder et al., 2000; Rentfrow et al., 2003)*, breeding system *(Högberg et al., 2001; Muriel et al., 2002)*, gender and castration *(Wood et al., 1989)*, age and weight at slaughtering *(Palanska et al., 1993; Bragagnolo and Rodríguez-Amaya, 2002)* and the genotype *(Gandemer et al., 1992)*. Additionaly, fatty acid composition of body fat depends also on the location in the carcass *(Delgado et al., 2002; Suzuki et al., 2006)* and considerable anatomical variation in fatty acid composition in the pig has been reported *(Franco et al, 2006)*.

The main objective of the present study was to characterize the fatty acid composition of the five different fat tissues in Swedish Landrace pig breed, as the most represented commercial pig breed in Serbia.

Materials and Methods

Trial was carried out on the farm, in slaughterhouse and laboratory of the Institute for Animal Husbandry (Belgrade, Serbia) on 8 male fatteners of Swedish Landrace breed. Pigs were fed *ad libitum* a standard diet for commercial slaughter pigs. The diet was based on silage corn, sunflower and soybean meal (Table 1).

Table 1. Composition of feed used in the final stage of fattening of pigs (from 60 to 103 kg body weight)

Mixture ingredients	(%)
Corn – silage	68.76
Livestock flour	15.00
Soybean meal	9.10
Sunflower meal	4.00
Chalk	1.40
Monocalcium phosphate	0.70
Salt	0.45
Premix	0.50
Synthetic lysine	0.09
TOTAL	100.00

All animals were slaughtered on the same day. Average pre-slaughter weight of fatteners was 103.3 kg. Animals were denied food 12h prior to slaughtering, but had free access to water. After slaughtering, pig carcasses were processed using standard techniques. After hair removal and evisseration, carcasses were cut into carcass sides and put in cooling chamber at temperature of 2-4°C for next 24 hours. Each left carcass side was disected in to main parts, and samples were taken from the lard, back area (subcutaneous fat), *M. Longissimus dorsi* (intramuscular fat), round (intermuscular fat) and belly (subcutaneous fat). Samples of fat tissues were vacuum-packed and frozen at -18°C until analysed.

The extraction of lipids from ground homogenate of samples was done with a mixture of chloroform and methanol (2:1 v/v) according to *Folch et al. (1957)*, whereupon the solvent was removed in evaporator until dryness. Fat extract was further used for fatty acids determination.

Fatty acid methyl esters (FAMEs) were prepared by transesterification by using 14% BF_3 in methanol (Sigma Aldrich, Germany), as described by *Thurnhofer and Vetter (2005)*. In brief, 20 mg of extracted fat was treated with 0.5 mL of methanolic KOH (0.5M) for 5 min/80°C. After cooling, 1.0 mL of methanolic BF_3 solution (14%) was added and heated for another 5 min/80°C. Then, the reaction vials were cooled in an ice bath (for 10 min), 2.0 mL of saturated sodium chloride solution and 2.0 mL of n-hexane were added, and the organic phase including the FAMEs was separated and subjected to GC analysis.

The GC instrument Shimadzu 2014 (Kyoto, Japan), used for FAMEs determination, was equipped with a split/splitless injector, fused silica cianopropyl HP-88 column (length 60 m, i.d. 0.25 mm, film thickness 0.20 μm) and flame ionization detector (FID). The column temperature was programmed. Injector and

detector temperature temperature was 260°C. The carrier gas was helium at a flow rate of 1.0 ml/min and injector split ratio of 1:110. Injected volume was 1 µl. The GC oven program started at 140°C (hold time 1 min), which then was raised at 4°C/min to 190°C, and at 3°C/min to 240°C (hold time 1 min). Chromatographic peaks in the samples were identified by comparing relative retention times of FAMEs peaks with peaks in a Supelco 37 Component FAMEs mix standard (Supelco, Bellefonte, USA) and the results are expressed as % of total fatty acids.

The total proportion of saturated fatty acids (SFA) was the sum of the weight percentages of caprylic (8:0), capric (10:0), lauric (12:0), myristic (14:0), palmitic (16:0), margaric (17:0) and stearic (18:0) acid. The total proportion of monounsaturated fatty acids (MUFA) was calculated by summing the weight percentages of palmitoleic (16:1 n-7), heptadecenoic (17:1), oleic (18:1 n-9), vaccenic (18:1 n-7) and gadoleic (20:1). Additionally, the total percentage of polyunsaturated fatty acids (PUFA) included linoleic (18:2 n-6), γ-linolenic (18:3 n-6), α-linolenic (18:3 n-3), eicosadienoic (20:2), eicosatrienoic (20:3 n-3), arachidonic (20:4 n-6) and adrenic (22:4 n-6) acid.

An analysis of variance (ANOVA) using the One-way ANOVA procedure of the SPSS 20.0 software (IBM SPSS Statistics, Version 20, IBM Corp, USA) was performed for all data considered. If the effect of main factor was found significant, Tukey-test was used to evaluate the significance of difference at $p<0.05$. All the data in are expressed as means ± standard deviation.

Results and Disscusion

Significant differences between samples were observed for the all measured fatty acids (Table 2), which is in agreement with findings of *Monziols et al. (2007).* In general, palmitic acid (16:0) was the most abundant SFA, with average percentages between 28.08 (intramuscular fat – INMF) and 22.54 (intermuscular fat – IMF), oleic acid (18:1 n-9) the most abundant MUFA (between 34.13% – INMF and 27.99% – IMF), and linoleic acid (18:2 n-6) the most abundant PUFA (between 27.14% – IMF and 12.17% – INMF).

INMF of *Longissimus* muscles showed a significantly ($p<0.05$) greater content of palmitic (16:0), palmitoleic (16:1 n-7), vaccenic (18:1 n-7) and arachidonic (20:4 n-6) acid and a lower content of caprylic (8:0), lauric (12:0), margaric (17:0), heptadecenoic (17:1), γ-linolenic (18:3 n-3), gadoleic (20:1) and eicosadienoic (20:2) acid, compared to other fat tissues. Linolenic acid (18:3 n-3) is the precursor fatty acid for the synthesis of eicosapentaenoic acid (EPA; 20:5 n- 3) and docosahexaenoic acid (DHA; 22:6 n-3), which play a major role in the control of cardiovascular diseases *(Conquer and Holub, 1998).* The greatest content of linoleic acid was found in intermuscular fat (IMF), with the addition of greatest

content of lauric (12:0), margaric (17:0), heptadecenoic (17:1) and γ-linolenic (18:3 n-3) acid ($p<0.05$).

Fat extracted from lard had the lowest content of capric (10:0), palmitoleic (16:1 n-7) and vaccenic (18:1 n-7) acid, but the greatest content of stearic (18:0) acid. Subcutaneous fat from back region had the lowest content of margaric (17:0), heptadecenoic (17:1) and arachidonic (20:4 n-6) acid, but also the greatest content of gadoleic (20:1) and adrenic (22:4 n-6) acid ($p<0.05$). Subcutaneous fat from belly had similar content of major fatty acids (16:0, 18:1 n-9, 18:2 n-6) as the fat extracted from back region, with the exception in a significantly higher content of stearic acid (18:0).

Results for linoleic acid (18:2 n-6) are in agreement with those of *Nguyen et al. (2003)*, that it is stored preferentially in adipose tissue rather than in muscle. However, in contrast with *Nguyen et al. (2003),* in our study, the highest content of linoleic acid is found in intermuscular fat, rather than in subcutaneous. In pigs, deposition of dietary fatty acids into tissue fat is preferential to endogenous synthesis and its largely reflects dietary fatty acid composition *(Hays and Preston, 1994; Moloney, 2002)*. In this regard, this difference could be due to a different quantity compositon of diets or the length of feeding.

Table 2. Fatty acid composition of the 5 fat tissue locations in Swedish Landrace pig

Fatty acid (%)	INMF[1]	IMF[2]	Lard	BSF[3]	Belly
8:0	ND[a4]	0.22 ± 0.02^{b}	0.07 ± 0.01^{c}	0.20 ± 0.02^{b}	0.10 ± 0.02^{c}
10:0	0.91 ± 0.04^{a}	0.90 ± 0.06^{a}	0.29 ± 0.02^{b}	0.72 ± 0.05^{c}	0.56 ± 0.02^{d}
12:0	ND[a]	0.36 ± 0.10^{b}	0.15 ± 0.01^{c}	0.18 ± 0.03^{c}	0.24 ± 0.06^{c}
14:0	2.95 ± 0.14^{a}	2.15 ± 0.10^{b}	1.71 ± 0.08^{c}	2.79 ± 0.17^{ab}	1.85 ± 0.15^{c}
16:0	28.08 ± 1.08^{a}	22.54 ± 1.34^{b}	25.49 ± 1.89^{c}	26.65 ± 1.11^{c}	24.89 ± 1.78^{bc}
16:1 n-7	3.73 ± 0.09^{a}	1.62 ± 0.08^{b}	1.12 ± 0.01^{c}	1.59 ± 0.01^{b}	1.56 ± 0.01^{b}
17:0	ND[a]	0.47 ± 0.02^{b}	0.31 ± 0.11^{c}	0.22 ± 0.04^{d}	0.34 ± 0.05^{c}
17:1	ND[a]	0.38 ± 0.06^{b}	0.21 ± 0.06^{c}	0.14 ± 0.02^{d}	0.26 ± 0.01^{c}
18:0	11.53 ± 0.91^{a}	10.26 ± 0.63^{a}	17.69 ± 0.73^{b}	12.81 ± 0.41^{a}	14.20 ± 0.44^{c}
18:1 n-9	34.13 ± 1.85^{a}	27.99 ± 1.15^{b}	30.40 ± 2.01^{b}	33.81 ± 2.14^{a}	32.41 ± 2.43^{a}
18:1 n-7	4.17 ± 0.42^{a}	1.86 ± 0.07^{b}	1.62 ± 0.09^{c}	1.87 ± 0.11^{b}	1.94 ± 0.04^{b}
18:2 n-6	12.70 ± 1.37^{a}	27.14 ± 2.91^{b}	17.57 ± 2.44^{c}	15.90 ± 2.59^{c}	18.16 ± 2.07^{c}
18:3 n-6	ND[a]	ND[a]	0.30 ± 0.06^{b}	0.22 ± 0.05^{b}	0.31 ± 0.03^{b}
18:3 n-3	0.60 ± 0.31^{a}	2.02 ± 0.42^{b}	1.23 ± 0.25^{c}	1.05 ± 0.33^{c}	1.23 ± 0.18^{c}
20:1	0.28 ± 0.23^{a}	0.51 ± 0.17^{b}	0.64 ± 0.16^{bd}	0.86 ± 0.14^{c}	0.70 ± 0.07^{d}
20: 2	0.37 ± 0.08^{a}	1.04 ± 0.06^{b}	0.86 ± 0.02^{b}	0.90 ± 0.07^{b}	0.86 ± 0.06^{b}
20:3 n-3	ND[a]	0.26 ± 0.01^{b}	0.19 ± 0.01^{b}	ND[a]	0.18 ± 0.02^{b}
20:4 n-6	0.56 ± 0.04^{a}	0.28 ± 0.02^{b}	0.17 ± 0.01^{c}	ND[d]	0.20 ± 0.02^{c}
22:4 n-6	ND[a]	ND[a]	ND[a]	0.08 ± 0.01^{b}	ND[a]

[1] INMF – Intramuscular fat; [2] IMF – Intermuscular fat; [3] BSF – Back-subcutaneous fat; [4] ND – not detected

[a-d]Different letters within the same row denote significant differences between means at $p<0.05$

Nutritionists recommend a reduction in total fat intake, particularly of SFA and trans fatty acids, which are associated with an increased risk of cardio-vascular diseases and some cancers *(Burlingame et al., 2009)*. Besides reducing fat intake, nutritionists urge consumers to increase their intake of PUFA, particularly the n-3 PUFA, at the expense of n-6 PUFA *(Simopoulos, 2004; Harris et al., 2009)*.

Statistical analysis of the results showed that the total saturated, monounsaturated and polyunsaturated fatty acid content was significantly different ($p<0.05$) in the five fat deposits (Figure 1). The lard fat showed the highest amount of SFA (45.69%) followed by the INMF and subcutaneous back and belly fat, while IMF had the lowest SFA content (36.90%). The higher melting point of the saturated fatty acids and the similar content of total SFA in INMF and subcutaneous fat could lead to the conclusion that the intramuscular fat have firm consistency. However, the consistency of the fat is determined not only by the degree of unsaturation and melting point of the fatty acids, but also by the amount and degree of maturation of the surrounding conjunctive tissue *(López-Bote and Rey, 2001)*. In this term, the most consistent fat is the subcutaneous fat, which is related to the greater amount and degree of maturation of the conjunctive tissue at this position on the carcass. The total MUFA showed a significantly higher percentage in the INMF (42.31%) than in other fat depots ($p<0.05$). The percentage of total PUFA was the highest in IMF (30.74%) and the lowest in INMF (14.22%).

Regarding nutritional recommendations for humans (<5), pork in general has too high ratio of n-6 and n-3 polyunsaturated fatty acids, primarily due to the high content of linoleic acid (18:2 n-6), which is found in high concentrations in corn *(Wood et al., 2003)*. The n-6/n-3 index was the highest in INMF (22.13), despite the lowest share of total n-6 fatty acids (13.25%), it had the lowest share of total n-3 fatty acids (0.60%), compared to other fat tissues (Figure 1). The highest share of total n-6 fatty acids was found in IMF (27.42%), but this is not reflected in the n-6/n-3 ratio.

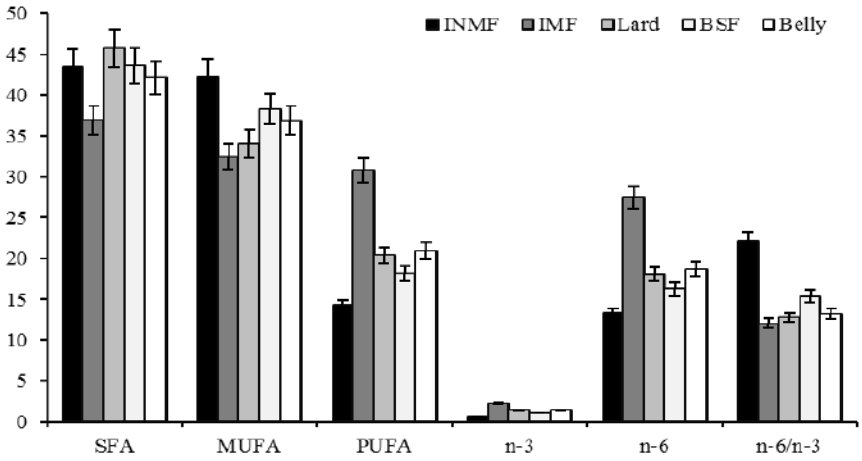

Figure 1. Total SFA, MUFA, PUFA, n-6 and n-3 fatty acid content and n-6/n-3 ratio of the 5 fat tissue locations in Swedish Landrace pig

Conclusion

The present data confirm that the various pig adipose tissues are different in their fatty acid composition. A higher percentage of PUFA, which are purportedly less harmful to human health, were measured in intermuscular fat, whereas this fat had significantly lower share of SFA compared to other fat tissues. The highest share of total n-6 fatty acids was found in INMF, but the highest n-6/n-3 ratio was found in IMF. Lard had the highest share of SFA, especially stearic acid. Subcutaneous fat from back and belly region had similar content of major fatty acids.

Poređenje sastava masnih kiselina u pet različitih masnih tkiva svinja rase Švedski Landras

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Rezime

Glavni cilj ovog istraživanja bio je da se uporedi sastav masnih kiselina masti u pet različitih masnih tkiva (uzetih iz sala, potkožnog masnog tkiva leđa,

intramuskularne masti iz *M. Longissimus dorsi*, intermuskularne masti iz buta i potkožnog masnog tkiva potrbušine) svinja rase švedski landras.

Značajno veći udeo ukupnih polinezasićenih masnih kiselina nađen je u intermuskularnoj masti (naročito veći udeo linolne (18:2 n-6) i γ-linoleinske kiseline (18:3 n-3)), u poredjenju sa drugim depoima masti ($p<0.05$). Sadržaj ukupnih masnih kiselina bio je najviši u salu, uglavnom zbog većeg udela stearinske kiseline (18:0). Najveći udeo ukupnih n-6 masnih kiselina nađen je u intermuskularnoj masti, dok je intramuskularna mast imala najviši n-6/n-3 odnos (što je u suprotnosti sa preporučenim prehrambenim vrednostima). Potkožna mast iz regiona leđa i potrbušine imala je sličan sadržaj glavnih masnih kiselina. Dobijeni rezultati potvrđuju da se različita adipozna tkiva svinja razlikuju po sastavu masnih kiselina.

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SALT REDUCTION IN MEAT PRODUCTS – BETTER AND HEALTHIER FUTURE

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Invited paper

Abstract: Salt is the primary source of sodium and increased consumption of sodium is associated with hypertension and increased risk of heart disease and stroke and several other disorders. Sodium intake is much more than recommended daily intake and often exceeds 5000 mg. American Heart Association recommends for persons with hypertension daily intake of not more than 1500 mg, and for persons with congestive heart disorders, daily sodium intake of not more than 1000 mg. Although salt has very important technological and antimicrobial role in meat processing, it is necessary to reduce salt and sodium content in meat products as well in food generally. There are many way to reduce the salt content in meat product, but it is not possible to make product without salt. Salt intake of less than 5 grams per day for adults helps to reduce blood pressure and risk of cardiovascular diseases, stroke and coronary heart attack. World Health Organization member states have agreed to reduce the global population's intake of salt by a relative 30% by 2025. An estimated 2.5 million deaths could be prevented each year if global salt consumption were reduced to the recommended level. Also, reducing salt intake has been identified as one of the most cost-effective measures countries can take to improve population health outcomes. In 2013, the World Health Assembly agreed 9 global voluntary targets for the prevention and control of NCDs, which include a halt to the rise in diabetes and obesity and a 30% relative reduction in the intake of salt by 2025. The "Global Action Plan for the Prevention and Control of Noncommunicable Diseases 2013-2020" gives guidance and a menu of policy options for Member States, WHO and other UN agencies to achieve the targets. In this paper are given some facts about role of salt in meat processing, about salt and sodium content in meat products and recommendations and global strategy for salt reducing in food.

Key words: Meat products, salt reduction, strategy, recommendations

Introduction

Salt (sodium chloride) was one of the first categories of trade/commercial exchange; also it has been subject of fees, taxes, caused wares and brought colonial power, created and crushed empires. Its history is ancient as the history of humans. Discovery and use of salt have made the food sustainable for longer period, available regardless of the season of the year and enabled its transport over long distances. It was important for all ancient peoples, particularly for Romans which controlled the salt price.

The oldest data on application of salt in the medicine date from 3000 b.c. and are associated with Egyptian builder and physician Imothepa, who stated that salt dried infected wound and can slow down inflammatory process, and implementation of salt in the human medicine is continued by Hypocrates in ancient Greece. Paracelsus introduces the salt as the third element, in addition to sulphur and mercury, and interrupts the dualist concept of alchemy and states than only well salted food can be digested properly. He is one of the first people to use salt baths in treatment of skin diseases.

For several millions of years, the prehistoric men consumed less than 0.5 g of salt daily *(Feng et al., 2010)*. Intentional adding of salt to food started around 5000-10000 years ago, at the beginning of the agricultural development and daily intake of salt reached average value of 10 g, which is in evolutionary sense relatively recent. Intake of kitchen salt is caused not only by physiological needs (athletes), but also habits which are acquired in the early childhood, as well as tradition in nutrition (region, i.e. climatic conditions, preparation of food, livestock resources, etc.). Of total daily amount of kitchen salt introduced into organism by common amounts of food (dishes prepared in the household, bread, bakery products, cheese, etc.), approx. 20% derives from meat products *(Wirth, 1991)*.

The highest consumption of salt was reached in 1870. With the advent of refrigeration and freezing, salt was no longer necessary in food preservation. And this lasted until 20th century when it was realized that higher income can be generated in production of salty products/food stuffs. Also, salt became the first functional food stuff by adding of iodine in prevention of goiter.

Increasing production of more and more processed food, rapid urbanization, and changing lifestyles are transforming dietary patterns. Highly processed foods are increasing in availability and becoming more affordable. People around the world are consuming more energy-dense foods that are high in saturated fats, trans fats, sugars, and salt.

Processed food has mostly high salt content (such as ready meals, processed meats like bacon, ham and salami, cheese, salty snack foods, and instant noodles,

among others). Also salt intake depends on the adding of salt cooking (bouillon and stock cubes) and on salting at the table (soy sauce, fish sauce and table salt).

Excessive sodium intake

Salt is the primary source of sodium and increased consumption of sodium is associated with hypertension and increased risk of heart disease and stroke. At the same time, as their eating patterns shift, people are consuming less fruit vegetables and dietary fibre (such as whole grains), that are key components of a healthy diet. Fruits and vegetables contain potassium, which contributes to reduce blood pressure.

Excessive intake of sodium and chloride, besides risk of heart diseases and stroke, also can lead to:

- Direct risk of heart attack *(Perry and Beevers, 1992)*,
- Hypertrophy of the left chamber *(Schmieder and Messerli, 2000)*,
- Sodium retention in extracellular fluid, i.e. water retention and clinical and idiopathic oedema, especially in women *(MacGregor and de Wardener, 1997)*,
- Increased hardness, i.e. decrease of elasticity of blood vessels, especially arteries, independent of the blood pressure *(Avolio et al., 1986)*,
- Proteinuria, primarily to urinary excretion of albumin, resulting in increased risk of heart and kidney diseases *(Du Cailar et al., 2002)*,
- Greater possibility of infection by *Helicobacter pylori* and risk of stomach cancer *(Tsugane et al., 2004)*,
- Increase of urinary excretion of calcium and risk of forming of kidney stones *(Cappuccio et al., 2000)*, also risk of reduced bone density, resulting in osteoporosis and compressive bone fractures, especially in case of women in menopause *(Devine et al., 1995)*,
- Exacerbations (more intensive and longer) asthmatic seizures *(Mickleborough et al., 2005)*,

- Increase of HOMA (homeostasis model assessment) insulin resistance in patients with essential hyper tension, majority of which have reduced glucose tolerance *(Kuroda et al., 1999)*, and

- Indirect incidence of obesity due to intensive intake of refreshing, non-alcoholic beverages *(Feng et al., 2010)*.

Human requirements in salt

Sodium from salt is mainly located in the extracellular fluid in the organism and it influences the maintaining of the water balance, nerve function, acid-base balance and muscle contractions. Although even unexpected, reduced intake of sodium can lead to muscle contractions, nausea, vomiting, anorection and coma. Because of this important role of sodium in the organism, requirements of humans in salt are often expressed as sodium requirements. According to some data, daily requirement in sodium for adults, to maintain metabolic processes and needs, is below 1500 mg. In case of athletes, requirements are higher, and even exceed 10000 mg per day, when large amount of sodium is lost through intensive sweating. However, daily intake of sodium is often over 5000 mg *(Benardot, 2012)*. American Heart Association recommends for persons with hyper tension daily intake of not more than 1500 mg, and for persons with congestive heart disorders, daily sodium intake of not more than 1000 mg.

Table 1 presents recommended and tolerable intake of sodium and chloride for infants, children, men and women, pregnant and nursing women.

Table 1 Recommended and tolerable daily intake of sodium and chloride *(Dietary Reference Intakes for Water, Potassium, Sodium, Chloride and Sulphate, 2004)*

	Recommended daily intake, g		**Tolerable daily intake, g**	
	Sodium	**Chloride**	**Sodium**	**Chloride**
Infants				
0-6 months	0.12	0.18	/	/
7-12 months	0.37	0.57	/	/
Children				
1-3 years	1.0	1.5	1.5	2.3
4-8 years	1.2	1.9	1.9	2.9
Males				
8-13 years	1.5	2.3	2.2	3.4
14-18 years	1.5	2.3	2.3	3.6
19-30 years	1.5	2.3	2.4	3.6
31-50 years	1.5	2.3	2.4	3.6
51-70 years	1.3	2.0	2.4	3.6

>70 years	1.2	1.8	2.3	3.6
Females				
8-13 years	1.5	2.3	2.2	3.4
14-18 years	1.5	2.3	2.3	3.6
19-30 years	1.5	2.3	2.4	3.6
31-50 years	1.5	2.3	2.4	3.6
51-70 years	1.3	2.0	2.4	3.6
>70 years	1.2	1.8	2.3	3.6
Pregnant women				
14-18 years	1.5	2.3	2.3	3.6
19-30 years	1.5	2.3	2.3	3.6
31-50 years	1.5	2.3	2.3	3.6
Breastfeeding women				
14-18 years	1.5	2.3	2.3	3.6
19-30 years	1.5	2.3	2.3	3.6
31-50 years	1.5	2.3	2.3	3.6

Functional and antimicrobial properties of salt in meat products

One of the major functions of salt in meat products is solubilisation of functional myofibril proteins, which activates the proteins to increase the hydration and water holding capacity (WHC) and, accordingly, improve the texture of product *(Ruusunen and Puolanne, 2005)*. According to *Hamm (1986)*, chlorine ions have the tendency to penetrate myofilaments causing their dissolution, whereas *Offer and Trinick (1983)* and *Offer and Knight (1988)* claim that sodium ions form ion "clod" around filaments. *Offer and Knight (1988)* base their hypothesis on selective bonding of chlorine ions to myofibrillar proteins. Dissolved myofibrillar proteins form sticky exudate on the surface of meat pieces which are subsequently connecting in this way during the heat treatment of the product. Matrix of proteins coagulated by heat tie in "trap" the free water. In emulsified meat products such as cooked sausages, dissolved proteins in form of continuous phase, represent the film around fat and water drops.

Inhibitory effect of salt on bacteria is based on lowering of the activity of water. At a certain concentration of kitchen salt, water exits the cells through osmosis, and this can slow down or completely stop the microbial development/growth. Relatively high concentrations of salt are necessary to inhibit microorganisms. Limit concentrations of sodium chloride for microbial growth are: 5% for *Clostridium botulinum* type E and *Pseudomonas fluorescens*, 6% for *Shigelae* and *Klebsiellae*, 8% for *Escherichia coli*, *Salmonellae*, *Bacillus cereus*, *C. botulinum* type A and *C. perfringens*, 10% for *C. botulinum* type B and *Vibrio parahaemolyticus*, 15% for *Bacillus subtilis* and *Streptococcaceae*, 18% for *Staphylococcus aureus*, 25% for *Penicillium* and *Aspregillus* species and 26% for

Halobacterium halobium, Bacterium prodigiosum and *Spirillium* species *(Prändl, 1988)*.

Content of salt in meat products

Content of salt in meat products depends, primarily, on technologically justified amounts, and, of course, on the influence of salt on the salinity flavour. There are numerous studies on content of salt in different meat products *(Vranić et al., 2009)*. The lowest content of salt is in the cooked sausages and meat cans. In cooked sausages salt content ranges from 1.28 to 2.03 g/100 g, in average 1.66 g/100 g; whereas in meat cans it ranges from 1.35 to 1.84 g/100 g, in average 1.67 g/100 g. In smoked meat products the content of salt is slightly higher and it ranges from 1.66 to 3.11 g/100 g, i.e. in average 2.19 g/100 g. In dry fermented sausages, technologically justified amount of salt is considerably higher and therefore 2.5-3.0% is added, since these products are subject to heat treatment, and salt serves for maintaining of the microbiological stability of the products. Content of salt in these sausages is 2.08-3.98 g/100 g, i.e. in average 2.61 g/100 g. The dry meat products have the highest content of salt. Due to long production process, i.e. brining, these products are salted or brined using 5-10% of salt or brine salt, in order to reduce, by action of the salt, the activity of water in order to prevent growth of undesirable microorganisms. Salt content in dry meat ranges from 3.78 to 7.35 g/100 g, in average 5.09 g/100 g.

Possibilities for reduction of salt content in meat products

Present trends in nutrition to reduce the content of sodium in meat products, as reported by *Ruusunen and Puolanne (2005)* and *Desmond (2006)*, can be achieved in the following way: (1) by reducing the amount of sodium chloride added *(Sofos, 1983; Lilić et al., 2008)*; (2) by substituting part of NaCl with other salts *(Sofos, 1983; Terell, 1983; Guàrdia et al., 2006; Lilić et al., 2008)*.; (3) by using flavour/aroma enhancers and masking agents *(Desmond, 2006)*; (4) combination of mentioned procedures *(Sofos, 1983; Terell, 1983)*; (5) adding of spice herbs and spice extracts to meat products *(Lilić and Matekalo-Sverak, 2007)*; (6) optimisation of the physical form of salt *(Angus et al., 2005)*; and (7) alternative process techniques *(Claus et Sørheim, 2006)*.

Potassium chloride is most common salt replacer, however, complete substitution of salt is not possible since, already in case of 50% substitution the bitter flavour is intensified and salinity is reduced. Use of potassium salts has often been disputed because of potential sensibility of one part of human population, such as persons suffering from diabetes type I, chronic renal insufficiency, last

stage of kidney diseases, persons with heart and adrenal insufficiency *(FSAI, 2005*). *US Department of Health and Human Services (2005)* indicate that diet rich in potassium weakens the effects of salt on blood pressure and daily potassium intake of 4.7 g is recommended.

In cooked hams, sodium chloride can be substituted with potassium chloride and 50% without any effect on sensory properties *(Frye et al., 1986)*. In hams, the use of 70% of NaCl and 30% of KCl, i.e. 70% NaCl and 30% $MgCl_2$, has no effect on smell, flavour/aroma, tenderness and overall impression compared to hams produced only using NaCl *(Collins, 1997)*.

In fermented sausages *(Gou et al., 1996)*, researchers have established absence of any difference in texture in substitution, but bitter flavour can be sensed already when 30% of KCl has been added. They also report that substitution of 40% with KCl and potassium lactate in dry meat does not lead to undesirable flavour characteristics.

According to *Ruusunen and Puolanne (2005)*, reduction of salt in fermented sausages is not possible below 2% due to inability to reach sufficiently low water activity which provides microbiological stability of these products.

Newer/more recent production processes are developed in a way that brine is injected into meat, and it contains KCl in combination with calcium citrate, calcium lactate, lactose, dextrose, potassium phosphate, ascorbic acid and sodium nitrite *(Riera et al., 1996)*.

Phosphates are also very successful in reduction of salt in products, although they act in synergy with sodium chloride. They increase the WHC by increasing the ion force when free groups of negative charge enable that proteins tie more water *(Trout and Schmidt, 1984)*. However, phosphates are also sodium carriers. So, sodium poly phosphate contains 31.24% of sodium, compared to 39.34% in sodium chloride, however its use is limited to approx. 0.5% in the product.

Ruusunen et al. (2002) established that the production of Bologna sausage and cooked ham with less salt (1.0-1.4%) is possible and that reduction of sodium content can be achieved by using potassium salts. It can be claimed that the use of phosphates in relation to sodium chloride is in the equivalent of 0.2% NaCl.

There is another possibility to compensate for technologically desirable properties of NaCl, and that is the use of ingredients such as fibres, hydrocolloids and starches which enable forming of the gel and protein coagulates *(Collins, 1997)*.

One of the possibilities for reduction of salt in meat products is the use of flavour enhancers and masking agents. There are many different commercial mixtures which usually contain yeast extracts, lactates, monosodium glutamate and nucleotides.

Flavour enhancers activate the receptors in the mouth/oral cavity and they compensate for the reduction of salt in the product *(Brandsma, 2006)*.

Certain authors *(Pasin et al., 1989)* have established that it is possible to reduce NaCl to 75% in cooked sausages, by combining KCl, preparation containing ribotide (commercial mixture of 5'-ribonucleotides IMP and GMP). Any addition of monosodium glutaminate leads to drop in the acceptability of the flavour of the product even by 50% due to incidence of bitter flavour caused by potassium salts. Linguagen, company from USA has patented a blocker of the bitter flavour, adenosine 5'-monophosphate, which blocks the activation of the gustducine in flavour receptor cells and accordingly, prevents stimulation of the flavour receptors *(McGregor, 2004)*. This blocker can be used to improve the flavour when combinations of KCl and NaCl are used.

There are several preparations on the market such as NeutralFres which removes the metallic, bitter flavour of KCl and gives the flavour similar to sodium salt, Magifique Salt-Away and Mimic, which mask the bitter and metallic character of KCl as well as SaltTrim.

Other combinations such as lysines and amber acid are used as substituents *(Turk, 1993)*. These substances have salty flavour and some antimicrobial and antioxidative properties and can be used as salt substitutes up to 75%. In regard to technological properties, i.e. water binding capacity, phosphates can be used, as well as starches and rubber.

Gou et al. (1996) studied the effect of glycine and potassium lactate as salt substituent and established that substitution of 40% of NaCl is possible using some of these compounds, which if used in larger amounts give unacceptably sweat flavour. In dry meat, substitution of up to 40% with potassium chloride and potassium lactate is possible without any significant difference in flavour, whereas 30% is maximum allowed amount if glycine is used as substituent.

There are also derivatives of mycoproteins (Mycoscent) which offer possibility to reduce the amount of sodium chloride by 50% in biscuits and snack foods and by 25% in hot, spicy dishes. Mycoscent 400 is natural source of ribonucleotide and glutamic acid, and it has flavour resembling broth and can be used to achieve the flavour of cooked meat in meat applications *(Mycoscent, 2005)*. Yeast autolysates are also known to suppress the bitter flavour of KCl, such as Provesta preparations, Aromild and Maxaromeselect. A problem with autolysates is their distinct broth flavour, which is not desirable in some products, and some of them have typical original umami flavour. By using certain technological procedures it is achieved that these preparations are optimized for meat products with neutral flavour and optimal umami effect.

Level of salinity depends also on physical form of salt. Salt flakes are proven to be functional in terms of binding, increasing pH, increasing protein

solubility in emulsion model systems *(Campbell, 1979)*. Salt flakes are better and faster soluble compared to granules, and this can be problem when in formulas no water is used, therefore flakes can be used for products where no water is added, for instance dry meat. Leatherhead Food International studied the optimization of the physical form of salt and monitored changes in the physical form of salt which is becoming more available and hence could be used in smaller amounts. This includes increase of its efficiency, change of the structure and modification of the perception of salt *(Angus et al., 2005)*.

Alternative process techniques include use of prerigor meat in manufacturing of meat products or use of high pressure technology *(Claus and Sørheim, 2006)*.

Salt reduction *(WHO Fact sheet N°393)*

Key facts

- High sodium consumption (>2 grams/day, equivalent to 5 g salt/day) and insufficient potassium intake (less than 3.5 grams/day) contribute to high blood pressure and increase the risk of heart disease and stroke.

- The main source of sodium in our diet is salt, although it can come from sodium glutamate, used as a condiment in many parts of the world.

- Most people consume too much salt—on average 9–12 grams per day, or around twice the recommended maximum level of intake.

- Salt intake of less than 5 grams per day for adults helps to reduce blood pressure and risk of cardiovascular disease, stroke and coronary heart attack. The principal benefit of lowering salt intake is a corresponding reduction in high blood pressure.

- WHO Member States have agreed to reduce the global population's intake of salt by a relative 30% by 2025.

- Reducing salt intake has been identified as one of the most cost-effective measures countries can take to improve population health outcomes. Key salt reduction measures will generate an extra year of healthy life for a cost that falls below the average annual income or gross domestic product per person.

- An estimated 2.5 million deaths could be prevented each year if global salt consumption were reduced to the recommended level.

Recommendations for salt reduction

- For adults: WHO recommends that adults consume less than 5 g (just under a teaspoon) of salt per day[1].
- For children: WHO recommends that the recommended maximum intake of salt for adults be adjusted downward for children aged two to 15 years based on their energy requirements relative to those of adults. This recommendation for children does not address the period of exclusive breastfeeding (0–6 months) or the period of complementary feeding with continued breastfeeding (6–24 months).
- All salt that is consumed should be iodized or "fortified" with iodine, which is essential for healthy brain development in the fetus and young child and optimizing people's mental function in general.

How to reduce salt in diets

Government policies and strategies should create environments that enable populations to consume adequate quantities of safe and nutritious foods that make up a healthy diet including low salt. Improving dietary habits is a societal as well as an individual responsibility. It demands a population-based, multisectoral, and culturally relevant approach.

Key broad strategies for salt reduction include:

- government policies - including appropriate fiscal policies and regulation to ensure food manufacturers and retailers produce healthier foods or make healthy products available and affordable;

- working with the private sector to improve the availability and accessibility of low-salt products;

- consumer awareness and empowerment of populations through social marketing and mobilization to raise awareness of the need to reduce salt intake consumption;

- creating an enabling environment for salt reduction through local policy interventions and the promotion of “healthy food” settings such as schools, workplaces, communities, and cities;

- monitoring of population salt intake, sources of salt in the diet and consumer knowledge, attitudes and behaviours relating to salt to inform policy decisions.

- Salt reduction programmes and programmes that promote fortification with micronutrients of salt, condiments or seasonings high in salt (bouillon cubes, soy and fish sauce) can complement each other.

Salt consumption at home can be reduced by:

- not adding salt during the preparation of food;

- not having a salt shaker on the table;

- limiting the consumption of salty snacks;

- choosing products with lower sodium content.

- Other local practical actions to reduce salt intake include:

- integrating salt reduction into the training curriculum of food handlers;

- removing salt shakers and soy sauce from tables in restaurants; Introducing product or shelf labels making it clear that certain products are high in sodium;

- providing targeted dietary advice to people visiting health facilities;

- advocating for people to limit their intake of products high in salt and advocating that they reduce the amount of salt used for cooking; and

- educating children and providing a supportive environment for children so that they start early with adopting low salt diets.

- Actions by the food industry should include:
- incrementally reducing salt in products over time so that consumers adapt to the taste and don't switch to alternative products;

- promoting the benefits of eating reduced salt foods through consumer awareness activities in food outlets;

- reducing salt in foods and meals served at restaurants and catering outlets and labelling sodium content of foods and meals.

WHO response

WHO guidelines on sodium and potassium provide thresholds for healthy intake. The guidelines also outline measures for improving diets and preventing NCDs in adults and children.

The "Global Strategy on Diet, Physical Activity and Health" was adopted in 2004 by the World Health Assembly (WHA). It calls on governments, WHO, international partners, the private sector and civil society to take action at global, regional and local levels to support healthy diets and physical activity.

In 2010, the WHA endorsed a set of recommendations on the marketing of foods and non-alcoholic beverages to children. These guide countries in designing new policies and strengthening existing ones to reduce the impact on children of the marketing of unhealthy food. WHO is also helping develop a nutrient profile model that countries can use as a tool to implement the marketing recommendations.

In 2011, world leaders committed to reducing people's exposure to unhealthy diets. The commitment was made through a Political Declaration of the High-level Meeting of the United Nations General Assembly on the Prevention and Control of NCDs.

In 2012, the WHA adopted six global nutrition targets, including the reduction of stunting, wasting and overweight in children, the improvement of breastfeeding and the reduction of anaemia and low birth weight.

In 2013, the WHA agreed 9 global voluntary targets for the prevention and control of NCDs, which include a halt to the rise in diabetes and obesity and a 30% relative reduction in the intake of salt by 2025. The "Global Action Plan for the Prevention and Control of Noncommunicable Diseases 2013-2020" gives guidance and a menu of policy options for Member States, WHO and other UN agencies to achieve the targets.

With many countries now seeing a rapid rise in obesity among infants and children, WHO in May 2014 set up a commission on childhood obesity. The Commission will draw up a report for 2015 specifying which approaches and actions are likely to be most effective in different contexts around the world.

Conclusion

In developed countries, approximately 80% of salt is added to food through different production stages. Many producers initiate salt reduction programs in their production and starting with reformulation of their products. WHO initiated the reduction strategy through regional directorates. Food industry in Republic of Serbia also can be included in this program and meat industry could have one of the main roles in this reduction program, contributing to the human health.

Several studies showed that reduced salt intake is economically justified. Reducing salt intake has been identified as one of the most cost-effective measures countries can take to improve population health outcomes. Key salt reduction measures will generate an extra year of healthy life for a cost that falls below the average annual income or gross domestic product per person. Some author have evaluated the effects and costs of the strategy to reduce the salt intake and tobacco control for 23 less developed and developing countries, and have proven that with the reduction of salt by 15% in the period 2006 to 2015, the death of 8.5 million people suffering from cardiovascular diseases could be prevented.

World Health Organization member states have agreed to reduce the global population's intake of salt by a relative 30% by 2025. An estimated 2.5 million deaths could be prevented each year if global salt consumption were reduced to the recommended level.

Meat industry all over the world has to take the active participation in the salt reduction and reformulation of meat products. This must become one of the goals in the social responsibility in human health improving and ensuring of better and healthier future.

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Rezime

So je primarni izvor natrijuma i povećana potrošnja natrijuma je povezana sa hipertenzijom i povećanim rizikom od srčanih oboljenja i moždanog udara, kao i nekoliko drugih poremećaja. Unos natrijuma je mnogo viši od preporučenog dnevnog unosa i često prelazi 5000 mg. American Heart Association preporučuje osobama sa hipertenzijom dnevni unos ne više od 1500 mg, a za osobe sa kongestivnim poremećajima srca, dnevni unos natrijuma ne više od 1000 mg. Iako so ima veoma važnu tehnološku i antimikrobnu ulogu u preradi mesa, neophodno je da se smanji sadržaj soli i natrijuma u proizvodima od mesa, kao i u hrani generalno. Postoji mnogo načina da se smanji sadržaj soli u mesnim proizvodima, ali nije moguće napraviti proizvod bez soli. Unos soli smanjen za 5 grama dnevno za odrasle pomaže da se smanji krvni pritisak i rizik od kardiovaskularnih bolesti, moždanog udara i koronarnog infarkta. Države članice Svetska zdravstvene organizacije su se dogovorile da smanje relativno globalni unos soli stanovništva za 30% do 2025. Procenjuje se da se 2,5 miliona smrtnih slučajeva može sprečiti svake godine ako se globalna potrošnja soli smanji do neophodnog nivoa. Takođe, smanjenje unosa soli je identifikovano kao jedan od najefikasnijih mera koje zemlje mogu preduzeti da se poboljša zdravstveni ishod stanovništva. U 2013., Svetska zdravstvena skupština je dogovorila 9 globalnih dobrovoljnih ciljeva za prevenciju i kontrolu nezaraznih bolesti, koje uključuju zaustavljanje rasta dijabetesa i gojaznosti i relativno smanjenje unosu soli od 30% do 2025. godine "Globalni akcioni plan za prevenciju i kontrolu nezaraznih bolesti 2013-2020 "daje smernice i meni političkih opcija za zemlje članice, SZO i druge agencije UN za postizanje ciljeva. U ovom radu su date neke činjenice o ulozi soli u preradi mesa, o sadržaju soli i natrijuma u proizvodima od mesa i preporukama i globalnoj strategiji za smanjenje soli u hrani.

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MINERAL LEVELS IN EDIBLE OFFAL FROM PIG

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Abstract: The aim of this paper was to provide an overview of the existing scientific literature on the mineral (K, P Na, Mg, Ca, Fe, Zn, Cu and Mn) levels in the raw edible offal from pig. According to *Serbian regulation (1985)*, the edible offal of a slaughtered pig that are removed in dressing include: tongue, heart, lungs, liver, spleen, kidney, brain, testicles, blood, part of stomach and colon, and intestines. Minerals are the inorganic elements other than carbon, hydrogen, oxygen and nitrogen, which remain behind in the ash when food is incinerated. They are usually divided into two groups – macrominerals (mainelements) and microminerals (trace elements). Main and trace elements have very varied functions (electrolytes, enzyme constituents, building materials in bones and teeth) in the human body. Also, they contribute to flavour, colour and texture of food.
A number of raw edible offal have considerable nutritional value, due to their low fat and energy contents and high levels of protein and micronutrients, including minerals and vitamins, all of which are essential for good health throughout life. Liver and spleen contain the greatest quantities of magnesium and iron, while liver contains the greatest quantities of zinc, copper and manganese. Spleen contains the highest amount of potassium. The highest content of sodium is for lungs and kidney. Spinal cord is the highest in phosphorus and calcium content.

Key words: pig, edible offal, mineral

Introduction

Animal by-products, or offal, include all parts of a live animal that are not part of the dressed carcass. Noncarcass material such as skin, blood, bones, meat trimmings, fatty tissues, feet, and internal organs of slaughtered pigs comprise a wide variety of products including human or pet food or processed materials in animal feed, fertilizer, or fuel (*Toldra et al., 2012*). In general, the total by-products range from 10 to 30% of the live weight of pig (*Ockerman and Basu, 2004*).
Offal fall into two categories and the divisions are edible and inedible. Biologically, most noncarcass material is edible if the product is cleaned, handled and processed appropriately. The yield of edible by-products from pig varies

tremendously depending on sex, live weight, fatness and methods of collection (*Ockerman and Hansen, 1988*; *Ockerman and Basu, 2004*).
Edible offal, sometimes called "variety meat" or "fancy meat", include the liver, heart, kidney, tongue, and other products (other organs, intestines and stomach) frequently used as edible by-products (*Ockerman and Hansen, 1988*; *Ockerman and Basu, 2004*). According to *Serbian regulation (1985)*, the edible offal of a slaughtered pig that are removed in dressing include: tongue, heart, lungs, liver, spleen, kidney, brain, testicles, blood, part of stomach and colon, and intestines.
While muscle foods are the more commonly consumed portion of an animal, edible by-products such as the entrails and internal organs are also widely consumed (*Toldra et al., 2012*). What is considered edible in one region may be considered inedible in another. Many factors influence the consumption of edible offal such as custom, religion, palatability and reputation. Edible offal are often different from skeletal tissue in structure, composition, functional and sensory properties (*Ockerman and Basu, 2004*).
Edible animal tissues (meat and offal) quality is the sum of all nutritive, sensoric, hygienic-toxicological and technological factors of meat. The nutritive factors of meat and edible offal quality include proteins and their composition, fats and their composition, vitamins, minerals, utilisation, digestibility and biological value (*Hofmann, 1990; Honikel, 1999*; *Olsson and Pickova, 2005; Tomović et al., 2008; Tomović et al., 2014a*). Edible offal in general possesses higher contents of some micronutrients, especially minerals and vitamins, than muscular tissue (*Ockerman and Basu, 2004*; *Lawrie and Ledward, 2006; Tomović et al., 2011b; Tomović et al., 2013a; Tomović et al., 2014b; Tomović et al., in press(a)*).
Minerals are the inorganic elements other than carbon, hydrogen, oxygen and nitrogen, which remain behind in the ash when food is incinerated. They are usually divided into two groups – macrominerals (mainelements) and microminerals (trace elements). Many of them are essential for plants, animals and humans (*Reilly, 2002*). Main functions of minerals in the human body are shown in Table 1 (*Food and Nutrition Board of the Institute of Medicine, National Academies, USA, 2015*).

Table 1. Main functions of minerals in the human body

Mineral	Function
K	Maintains fluid volume inside/outside of cells and thus normal cell function; acts to blunt the rise of blood pressure in response to excess sodium intake, and decrease markers of bone turnover and recurrence of kidney stones
P	Maintenance of pH, storage and transfer of energy and nucleotide synthesis
Na	Maintains fluid volume outside of cells and thus normal cell function
Mg	Cofactor for enzyme systems
Ca	Essential role in blood clotting, muscle contraction, nerve transmission, and bone and tooth formation
Fe	Component of hemoglobin and numerous enzymes; prevents microcytic hypochromic anemia
Zn	Component of multiple enzymes and proteins; involved in the regulation of gene expression
Cu	Component of enzymes in iron metabolism
Mn	Involved in the formation of bone, as well as in enzymes involved in amino acid, cholesterol, and carbohydrate metabolism

The nutrient levels in foods are variable. The major sources of variability in nutrient composition are the wide diversity of soil and climatic conditions (geographical origin), seasonal variations, physiological state and maturity, as well as cultivar and breed (*Greenfield and Southgate, 2003*). The continuous innovations in the breeding systems, rearing practices, feeds composition, changes in slaughtering methods and ageing, largely contribute to induced changes in the concentration of some micronutrients in animal tissue (*Lombardi-Boccia et al., 2005*; *Greenfield et al., 2009*). According to *Greenfield and Southgate (2003),* edible animal tissues, exhibits natural variations in the amounts of nutrients contained, and the limits of the natural nutrient variations are not defined.
Changes in the mineral content also usually occur in the processing of raw materials, e. g., in thermal processes and material separations (*Belitz et al., 2009; Tomović et al., in press(b)*)
On the other hand, edible animal tissues (meat and offal) are not only the elementary sources of nutrients, but may also contain chemical substances with toxic properties (*Dabrowski, 2004*). Many of the toxic chemicals are efficiently retained in the edible offal, especially in liver and kidney (*Tomović et al., 2011a, 2011c; Jokanović et al., 2013; Tomović et al., 2013b*).

Mineral composition of raw edible offal from pig

Corresponding data for the mineral (potassium - K, phosphorous - P, sodium - Na, magnesium - Mg, calcium - Ca, iron - Fe, zinc - Zn, copper - Cu and manganese - Mn) content of various edible offal tissues (tongue, heart, lungs, liver, spleen, kidney, brain and spinal cord) are shown in Table 2. Among the raw edible offal, potassium is the most abundant mineral. Potassium is the highest for spleen (396–464 mg/100g), followed by spinal cord (377 mg/100g), brain (258–388 mg/100g), liver (217–370 mg/100g), heart (240–300 mg/100g), tongue (243–282 mg/100g), lungs (223–303 mg/100g) and kidney (189–290 mg/100g).
The second most abundant mineral in raw edible offal is phosphorus. With the exception of spinal cord, with phosphorus content of 501 mg/100g (only one data), the phosphorus content is highest in liver, where it ranges from 288 mg/100g to 430 mg/100g. Brain is next highest in phosphorus content (282–363 mg/100g), followed by spleen (205–301 mg/100g), kidney (204–287 mg/100g), lungs (196–221 mg/100g), heart (160–245 mg/100g) and tongue (173–195 mg/100g).
Furthermore, kidney (121–190 mg/100g) and lungs (153–163 mg/100g) are the highest in sodium content. The content of sodium in spinal cord is 146 mg/100g, while in brain it ranges from 120 mg/100g to 158 mg/100g. Sodium is lowest in spleen (83–130 mg/100g), tongue (87.7–110 mg/100g), liver (80–150 mg/100g) and heart (56–107 mg/100g).
Contents of magnesium are similar for spleen (13–34 mg/100g), liver (18–25.7 mg/100g), heart (17–25 mg/100g), kidney (17–23.3 mg/100g) and tongue (17.4–23 mg/100g). The content of magnesium in lungs and brain ranges from 13.8 mg/100g

to 17 mg/100g and from 10 mg/100g to 20 mg/100g, respectively. Spinal cord is the lowest in magnesium content (8.3 mg/100g).
For a given raw edible offal, spinal cord is markedly the highest in calcium content (26 mg/100g). Other raw edible offal (brain: 10–18.7 mg/100g; heart: 5–35 mg/100g; tongue: 6.52–16 mg/100g; lungs: 5.8–20.8 mg/100g; kidney: 6.97–20.2 mg/100g; liver: 5–20.4 mg/100g; spleen: 5.4–10 mg/100g) do not differ markedly in calcium content. There is, however, a wide variation in the calcium content for each type of raw edible offal.

Table 2. Mineral content (mg/100g) of raw edible offal tissues

Source	Edible offal	K	P	Na	Mg	Ca	Fe	Zn	Cu	Mn
Denmark – Danish food composition database (2009)	Tongue	282	195	92	18	6.52	2.15	2.6	0.23	0.034
	Heart	298	204	107	21	5.29	6.0	2.1	0.41	0.038
	Liver	271	369	80	18	6.48	13.4	6.78	1.01	0.39
	Kidney	244	238	160	18	6.97	3.3	2.59	0.715	0.15
Finland – Fineli - Finnish food composition database (2013)	Liver	370	430	150	24	6.0	31.4	9.0		
	Kidney	230	260	165	18	9	8.4	2.3		
Italy – Food composition database for epidemiological studies in Italy (2008)	Heart	300	245	80	25	35	5.3	2.3	0.47	0.03
	Liver	356	362	108	21	10	18.0	6.3	2.6	0.29
Lawrie and Ledward (2006)	Liver	320	370	87	21	6	21.0	6.9	2.7	
	Kidney	290	270	190	19	8	5.0	2.6	0.8	
	Brain	270	340	140	15	12	1.6	1.2	0.3	
Norway – Norwegian food composition tables (2014)	Heart	240	160	95	17	6	5.4	1.3		
	Liver	287	411	94	20	5	18.7	8.7	0.64	
Canada – Canadian nutrient file (2012); USA – USDA national nutrient database for standard reference (2011)	Tongue	243	193	110	18	16	3.35	3.01	0.070	0.011
	Heart	294	169	56	19	5	4.68	2.8	0.408	0.063
	Lungs	303	196	153	14	7	18.90	2.03	0.083	0.017
	Liver	273	288	87	18	9	23.3	5.76	0.68	0.34
	Spleen	396	260	98	13	10	22.32	2.54	0.131	0.072
	Kidney	229	204	121	17	9	4.89	2.75	0.62	0.12
	Brain	258	282	120	14	10	1.6	1.27	0.24	0.094
Czech Republic – Czech food composition database (2013); Slovak Republic – Slovak food composition database (2015)	Tongue	258	192	104	23	11	4.3	2.6		
	Heart	287	184	104	19	17	4.7	2.04	0.62	
	Lungs	242	221	163	17	5.8	9.1	0.7	0.1	
	Liver	317	353	115	24	8	15.3	6.6	2.6	
	Spleen	401	205	130	34	9	19.8	2.6	0.2	
	Kidney	249	260	184	21	8.5	6.8	1.7	0.5	
	Brain	318	363	158	20	14.1	3.4	1.6	0.4	
Serbia – Tomović et al. (2011b); Tomović et al. (2013a)	Liver	217	383	82.2	25.7	20.4	21.8	9.82	1.61	0.35
	Kidney	189	287	130	23.3	20.2	7.42	2.99	1.29	0.22
Serbia – Tomović et al. (in press(a))	Tongue	246	173	87.7	17.4	12.8	2.56	2.13	0.24	0.036
	Heart	285	171	93.0	20.0	8.5	4.48	1.61	0.32	0.035
	Lungs	223	209	158.4	13.8	20.8	6.37	2.09	0.12	0.033
	Liver	359	345	80.2	20.4	13.6	33.58	5.34	0.37	0.267
	Spleen	464	301	83.0	18.0	5.4	27.49	3.15	0.20	0.045
	Kidney	248	226	157.2	19.1	13.0	5.96	2.24	0.39	0.123
	Brain	388	354	142.3	10.0	18.7	3.82	1.56	0.32	0.048
	Spinal cord	377	501	146.0	8.3	26.0	1.78	0.67	0.21	0.045

Spleen and liver are the best sources of iron. Values range from 19.8 mg/100g to 27.49 mg/100g for spleen and from 13.4 mg/100g to 33.58 mg/100g for liver. Also, lungs are the rich source of iron, with a content ranging from 6.37 mg/100g to 18.9 mg/100g. Iron values are similar for kidney (3.3–8.4 mg/100g) and heart (4.48–6 mg/100g) and for tongue (2.15–4.3 mg/100g) and brain (1.6–3.82 mg/100g). The lowest amount of iron is for spinal cord (1.78 mg/100g).
Liver is the richest source of zinc. Values range from 5.34 mg/100g to 9.82 mg/100g for liver. Values for 100 g spleen, tongue, kidney, heart, lungs and brain were in the ranges: 2.54–3.15 mg/100g, 2.13–3.01 mg/100g, 1.7–2.99 mg/100g, 1.3–2.8 mg/100g, 0.7–2.09 mg/100g and 1.2–1.6 mg/100g. Spinal cord is the lowest in zinc content (0.67 mg/100g).
Copper is the highest for liver (0.37–2.7 mg/100g). Otherwise, it is in the range: 0.39–1.29 mg/100g (kidney), 0.32–0.62 mg/100g (heart), 0.3–0.4 mg/100g (brain), 0.21 mg/100g (spinal cord), 0.07–023 mg/100g (tongue), 0.131–0.2 mg/100g (spleen) and 0.083–0.12 mg/100g (lungs).
For a number of raw edible offal listed, no values for manganese are available. Of the raw edible offal with values for manganese, liver and kidney contain the highest amounts. A content ranges from 0.267 mg/100g to 0.39 mg/100g in liver and from 0.12 mg/100g to 0.22 mg/100g in kidney. The remaining raw edible offal contain less than 0.1 mg/100g manganese. Manganese levels in brain, spleen, spinal cord, heart, tongue and lungs range from 0.011 mg/100g (tongue) to 0.094 mg/100g (brain).

Dietary reference intakes for minerals

Dietary reference intake is the general term for a set of reference values used to plan and assess nutrient intakes of healthy people. Health authorities in most countries have established recommendation for daily intake levels of essential minerals. Dietary reference intake for minerals (K, P Na, Mg, Ca, Fe, Zn, Cu and Mn) recommended by the *Food and Nutrition Board of the Institute of Medicine, National Academy of Sciences, USA,* (*2015*) are shown in Table 3.

Table 3. Dietary reference intake for minerals

	K (g/d)	P (mg/d)	Na (g/d)	Mg (mg/d)	Ca (mg/d)	Fe (mg/d)	Zn (mg/d)	Cu (µg/d)	Mn (mg/d)
Infants									
0−6 months	0.4	100	0.12	30	200	0.27	2	200	0.003
7−12 months	0.7	275	0.37	75	260	11	3	220	0.6
Children									
1−3 years	3.0	460	1.0	80	700	7	3	340	1.2
4−8 years	3.8	500	1.2	130	1000	10	5	440	1.5
Males/Females									
9−13 years	4.5	1250	1.5	240	1300	8	8	700	1.9/1.6
14−18 years	4.7	1250	1.5	410/360	1300	11/15	11/9	890	2.2/1.6
19−30 years	4.7	700	1.5	400/310	1000	8/18	11/8	900	2.3/1.8
31−50 years	4.7	700	1.5	420/320	1000	8/18	11/8	900	2.3/1.8
51−70 years	4.7	700	1.3	420/320	1000/1200	8/8	11/8	900	2.3/1.8
> 70 years	4.7	700	1.2	420/320	1200	8/8	11/8	900	2.3/1.8
Pregnancy									
14−18 years	4.7		1.5		1300				
19−50 years	4.7		1.5		1000				
≤ 18 years		1250		400		27	12	1000	2.0
19−30 years		700		350		27	11	1000	2.0
31−50 years		700		360		27	11	1000	2.0
Lactation									
14−18 years	5.1		1.5		1300				
19−50 years	5.1		1.5		1000				
≤ 18 years		1250		360		10	13	1300	2.6
19−30 years		700		310		9	12	1300	2.6
31−50 years		700		320		9	12	1300	2.6

Edible offal from pig as human food

Edible offal offer a range of foods which are nutritionally attractive and have a wide variety of sensory properties. The structure of the edible offal clearly influences the possible uses of these products. Brains are usually prepared for the table rather than for use in manufactured meat products. Because of soft texture, brains are blanched to firm the tissue before proceeding with other cooking methods. Hearts are used as table meats. Whole heart can be stuffed in a variety of ways and roasted or braised. Sliced heart meat is grilled or sautéd. Heart meat is also used in sausages and manufactured meats. Kidney are used whole or sliced, and generally either grilled, sautéd or braised, but they are not used to any extent in manufactured meat products. Liver is the most widely used edible offal and is used in many types of manufactured meat products. Livers from pigs are better suited to manufactured meats, particularly liver sausages paté because they have a strong

flavour. Pig lungs are mainly used to make stuffings and some types of sausages and manufactured meat. They have limited other uses but may be braised either whole or cubed. Spleens are minced and used in stuffings or manufactured meats. Tongues are used fresh or salted and generally boiled or braised, as well as may be canned (*Ognjanović et al., 1985; Spooncer, 1988*).

Conclusion

The present paper provides mineral content on the raw edible offal from pigs. The mineral composition (K, P Na, Mg, Ca, Fe, Zn, Cu and Mn) was significantly influenced by the type of edible offal (tongue, heart, lungs, liver, spleen, kidney, brain and spinal cord). Based on presented data, consumer knowledge, mainly of the mineral content of specific edible offal, could be increased. Additionally, more studies are needed to provide a better knowledge about raw edible offal characteristics, especially including eating quality of some cooked edible offal.

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Sadržaj minerala u jestivim iznutricama svinja

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Rezime

Cilj ovog rada je da na osnovu postojeće naučne literature prikaže sadržaj minerala (K, P Na, Mg, Ca, Fe, Zn, Cu i Mn) u svežim jestivim iznutricama svinja.
Prema *Regulativi u Srbiji (1985)*, pod jestivim iznutricama zaklanih svinja podrazumevaju se: jezik, srce, pluća, jetra, slezina, bubrezi, mozak, beli bubrezi (testisi), krv, delovi želuca i debelog creva i tanka creva.

Minerali su neorganski elementi, osim ugljenika, vodonika, kiseonika i azota, koji ostaju u pepelu nakon spaljivanja hrane. Oni se najčešće dele u dve grupe – makrominerali (glavni elementi) i mikrominerali (elementi u tragovima). Glavni i elementi u tragovima imaju veoma različite funkcije (elektroliti, ulaze u sastav enzima, gradivni materijal u kostima i zubima) u organizmu ljudi. Takođe, oni doprinose aromi, boji i teksturi hrane.

Jestive iznutrice, uglavnom, imaju značajnu nutritivnu vrednost, zbog niskog sadržaja masti i niske energetske vrednosti, kao i zbog visokog sadržaja proteina i mikronutrijenata, uključujući minerale i vitamine, koji su od suštinskog značaja za dobro zdravlje tokom života. Magnezijum i gvožđe su najzastupljeniji u jetri i slezini, dok su cink, bakar i mangan najzastupljeniji u jetri. Kalijum je nazastupljeniji u slezini, a natrijum u plućima i bubrezima. Od svih prikazanih iznutrica, fosfor i kalcijum su najzastupljeniji u kičmenoj moždini.

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RELIABILITY OF THE PIGLOG ASSESSMENT OF MEATINESS AND CORRELATION OF CARCASS QUALITY PROPERTIES

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Invited paper

Abstract: The aim of the work was to determine the reliability of measurement using PIGLOG 105 of fat thickness (SL1 and SL2), the depth of *Musculus longissimus* (ML) and to estimate the meatiness in living and slaughtered animals (dissection on the slaughter line according to the Ordinance of R. Serbia). The study was conducted on 170 animals of two genotypes: Swedish Landrace (n = 73) and F_1 crosses SLxLY (n = 97), of both sexes (castrated males n = 79 and females n = 91). The average fat thickness was determined at an average body mass of 109.6 kg using PIGLOG 105 - SL1 = 14.55 mm, SL2 = 12.74 mm, while the thickness of the fat was determined by dissection, and the following values were determined on the same locations: SL1 = 16.42 mm, SL2 = 14.19 mm. Depth of ML established using PIGLOG 105 was 47.59 mm, while the greater depth of ML was obtained by dissection (55.58 mm). Percentage and meat yield determined with PIGLOG 105, dissection and JUS were 55.77%; 57.72% and 49.36 kg and 44.42% and 38.64 kg, respectively. Based on these results obtained, we can see that JUS has significantly lower absolute values for percentage and yield of meat compared to PIGLOG 105 and dissection. It was found that there is a statistically significant difference ($P<0.01$) between the estimated meatiness values. Correlations between traits measured using PIGLOG 105 and the same values determined on dissected carcass sides are as follows: strong for SL1 (r = 0.724), very strong for SL2 (r = 0.820), and medium for the depth of ML (r = 0.447). The correlation for the percentage of meat is very strong (r = 0.787). There were statistically highly significant ($P <0.01$) correlations for the meatiness estimated using PIGLOG 105 and dissection.

Keywords: pigs, PigLog 105, lean meat content, carcass quality, correlation

Introduction

Pig breeding/production has a very important role in the production of food of animal origin because pork is of great importance both for human consumption and for processing into products of higher level of processing (*Radović et al., 2011; 2013a*). Demand for pork is constantly growing, because of the total requirements for animal proteins in the human diet over 50% is covered by this kind of meat. The first standards for assessing the quality of pig carcasses/carcass sides were defined at the end of the sixties, and before the end of the nineties, in most EU countries, meatiness/lean meat content evaluation was based on measurements of back fat thickness. The basis for such a valuation method is a high correlation (r = 0.75) between the thickness of backfat and lean meat content in carcasses/carcass sides. In recent years, a significant part of scientific research is focused on finding the optimal solution for fast and reliable quality assessment of pig carcasses *in vivo* and *in vitro*. Individual quantitative traits of pigs are unequally inherited, which means that the possibilities for their improvement by selection and breeding are different. Heritability coefficients for fattening and carcass quality traits are moderate to high (*Knapp et al., 1997; Radović et al., 2003*). Improved selection for the content of meat has caused significant reduction of subcutaneous fat, perhaps even more of intramuscular fat content (*Bahelka et al., 2007*). Payment to pig producers based on meat yield would motivate the producers of fattening pigs, i.e. enables them to realize a significantly higher price for pigs produced, adequate compensation for quality selection/breeding, choice of food, optimal finalization of fattening ... (*Okanović et al., 2008*). Based on the above said, we can perceive the importance and great responsibility of selection in assessing the breeding value, and permanent positive impact on the changes in the herd and thus increased production.

Bearing in mind the above mentioned, the aim of this study was to determine the accuracy of measurements of back fat thickness and precision of *in vivo* evaluation of meatiness/lean meat content and to emphasize the necessity of amendments to the *Regulation on quality of slaughtered pigs and pork categorization (OJ SFRY, 1985).*

Material and methods

In order to determine the reliability of measurement using PIGLOG 105, fat thickness (SL1 and SL2) and the depth of *Musculus longissimus* (ML) were measured on live and slaughtered animals. The study was conducted on 170 animals of two genotypes: Swedish Landrace (n = 73) and F_1 crosses SLxLY (n = 97), of both sexes (castrated males n = 79 and females n = 91). The measuring of the thickness of the fat in the loin section (SL1) was performed using ultrasound apparatus (PIGLOG 105), between the 3rd and 4th lumbar vertebra (measured from the last lumbar vertebra), 7 cm lateral to the back line. The second measurement of back fat thickness in the lumbar part (SL2) and depth of ML was taken between the 3rd and 4th rib from the back, 7 cm lateral to the back line.

Places for measuring of fat thickness and ML depth were previously marked by tattooing on live animals. After chilling of carcass sides, fat thickness was measured (SL1 and SL2) and ML depth in order to determine the correlation between measures taken on live animals and carcass sides. The back fat thickness and depth ML were measured on animals of live mass from 90 kg to 120 kg. In order to determine the reliability of estimates of lean meat content using PIGLOG 105, 170 animals were dissected according to the method of *Weniger et al. (1963).* Slaughtering of pigs and dissection were performed in the experimental slaughterhouse of the Institute for Animal Husbandry, Belgrade. To determine the yield (kg) and the share of meat (%) in the carcass sides (JUS), on the basis of measurements of warm carcass sides and the sum of the thickness of fat on the back (back fat in the middle of the back and the second measure of back fat thickness in the area where *m. gluteus medius* grows into adipose tissue) tables for meaty pigs are used, which are an integral part of the *Regulation (1985).*

The measurements were made on the slaughter line and a third at the beginning of said muscle - cranial).

Genetic correlation (r_G) were calculated by using the formula:

$$r_G = \frac{Cov}{\sqrt{\sigma^2_{IZ} * \sigma^2_{UN}}}$$

where:
rg = genetic correlations
σ^2_{IZ} = variance between groups (sires)
σ^2_{UN} = variance within groups (sires)
Cov = covariance

The strength of the correlation between the properties is defined by Roemer-Orphalov scale of classification (quote - *Latinović, 1996*). Testing of zero hypothesis that there is no correlation between the two properties at the population level, was carried out based on the tables of critical values of *t* distribution for the level of significance of 5 and 1 % and degree of freedom n-2 (*Snidikor and Cochran, 1971*).

Results and Discussion

Table 1 shows the average values and the variability of indicators of meatiness/lean meat content obtained when processing data using PIGLOG 105, dissection and JUS. The average body mass of the examined pigs was 109.6 kg, while the mass of warm carcass side was 87.69 kg. The average fat thickness determined by PIGLOG 105 was SL1 = 14.55 mm, SL2 = 12.74 mm, while the thickness of the fat determined by dissection on the same places was SL1 = 16.42 mm, SL2 = 14.19 mm. Depth of ML established by PIGLOG 105 is 47.59 mm, while the depth of ML determined by dissection was greater (55.58 mm). Share and yield of meat determined using the PIGLOG 105, dissection and JUS was 55.77%; 57.72% and 49.36 kg, and 44.42% and 38.64 kg, respectively. Based on these results, we see that according to JUS, absolute values were significantly lower for share and yield of meat in relation to PIGLOG 105 and dissection. Results in Table 1 show significant absolute differences in carcass quality traits determined by PIGLOG 105, dissection and according to the applicable *Regulation (1985).* Based on the average values obtained using PIGLOG 105 and dissection for SL1 = 14.55 and 16.42 mm, SL2 = 12.74 and 14.19 mm, respectively, ML = 47.59 mm and 55.58 mm, and the percentage of meat = 55.77% and 57.72%, identified differences were highly statistically significant ($P<0.01$). The established values for fat thickness by ultrasound device Piglog 105 in our study were in absolute vale significantly higher (SL1 and SL2 14.55 mm 12.74 mm in relation to research by *Gogić et al. (2013)* for the same genotypes (SL1 and SL2 12.98 mm 27.11 mm), while for the depth and the estimated lean meat content lower values were established (47.59 mm 50.72 mm and 55.7% : 58.8%, respectively). Also higher estimated lean meat content in relation to our result for the four genotypes was determined in research of *Tyra et al. (2006).* These authors found similar estimation of the lean meat content by Piglog 105 and Aloka SSD 500 (57.1% and 57.7%) and slightly higher value by dissection of 60.6%. Approximately similar value of estimated meatiness/lean meat content by PIGLOG 105 was determined in the study of *Tyra et al. (2011)* according to the genotypes Large White (55.8%) and Landrace (56.1%) which were also subject of our study (55.77%), while the

estimated lean meat content by partial dissection was higher than in our study for the mentioned genotypes (0.9% and 1.0%).

Table 1. Average values and variability of indicators of meatiness/lean meat content

Properties	$\overline{x}$	SD	CV
Body mass, kg	109.60	10.57	9.64
Mass of warm carcass sides (both), kg	87.69	8.97	10.23
PIGLOG 105			
SL1, mm	14.55	2.45	16.84
SL2, mm	12.74	2.32	18.21
ML, mm	47.59	5.16	10.84
Meat share, %	55.77	2.34	4.19
DISSECTION			
SL1, mm	16.42	3.95	24.06
SL2, mm	14.19	3.56	25.09
ML, mm	55.58	6.13	11.03
Meat share, %	57.72	2.89	5.01
Meat, kg	49.36	4.38	8.87
JUS			
Back fat – loin, mm	16.10	3.34	20.74
Back fat – back, mm	17.58	2.82	16.04
Meat share, %	44.42	2.78	6.25
Meat, kg	38.64	3.76	9.73

Table 2 shows the significance of differences for meat percentage (%) and meat yield (kg) tested by t-test between PIGLOG 105 and JUS, dissection and PIGLOG 105 as well as dissection and JUS. On the basis of these results statistically significant difference ($P<0.01$) between the estimated meatiness values was determined. Significant differences in the estimated meatiness/lean meat content between JUS and partial dissection (44.23% : 60.69%) was found in the study of *Radovic et al. (2012).* In an identical experiment *Radovic et al. (2013b)* have found significantly higher values for fat thickness SL1 and SL2 and lower values for the depth of the ML and the leanness estimated using PIGLOG, dissection and JUS method compared to the repeated research.

Table 2. The significance of differences in meatiness/lean meat content (%, kg) between PIGLOG 105: JUS, Dissection: PIGLOG 105 and Dissection: JUS

Properties	PIGLOG 105	JUS	t-test
	$\overline{X}$	$\overline{X}$	
Meat share, %	55.77	44.42	**
Properties	DISEKCIJA	PIGLOG 105	t-test
	$\overline{X}$	$\overline{X}$	
Meat share, %	57.72	55.77	**
Properties	DISEKCIJA	JUS	t-test
	$\overline{X}$	$\overline{X}$	
Meat share, %	57.72	44.42	**
Meat, kg	49.36	38.64	**

** ⇨ P<0.01; * ⇨ P<0.05; NS ⇨ P>0.05;

Table 3 shows the degree of dependence between PIGLOG 105, dissection and JUS for meatiness/lean meat content indicators. By examining the correlations between traits measured using PIGLOG 105 and the same traits determined by dissection, it can be seen that the correlation for SL1 was strong (r = 0.724), for SL2 very strong (r = 0.820), and medium for the depth of ML (r = 0.447). The correlation between the percentage of meat is very strong (r = 0.787). Very strong correlation between SL1 and SL2 (r = 0.812) was found in measurements of these properties obtained by dissection compared with the same properties that were measured using PIGLOG 105, where the correlation was somewhat weaker (r = 0.708). When it comes to genetic correlations between body fat i.e. fat thickness and meatiness/lean meat content, in the research of *Sonesson et al. (1998)* a strong negative correlation (r_G = -0.77) between fat thickness and meatiness/lean meat content was established. The study by *van Wijk et al. (2005)* reveales the absolute negative genetic correlation between fat thickness and share of meat (r_G=-0,98) obtained using the assessment with Hennessy grading probe. The results of our study confirmed the results of *Groeneveld et al. (1998)*, who identified a genetic correlation of 0.60 to 0.82% for meat measured using PIGLOG 105 and dissection. Based on the tested level of significance according to *Snedecor and Cochrann (1971)* and given values it can be seen that all of these correlations were statistically highly significant (P<0.01). On the basis of these results we can see that the stronger correlation is determined for SL2 in comparison with SL1. Also, it can be seen that the correlation between PIGLOG-105 and dissection for meatiness/lean meat content is stronger, compared to the same trait between dissection and JUS.

Table 3. Correlation between Piglog 105, dissection and JUS indicators for meatiness/lean meat content

Properties	PIGLOG 105				DISSECTION					JUS	
	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	Meatiness/lean meat content, kg (13)
B. mass - live (1)	0.479**	0.436**	0.237**	-0.471**	0.469**	0.418**	0.167*	-0.249**	0.772**	-0.419**	0.802**
Mass of cooled carcass sides - both(2)	0.502**	0.446**	0.298**v	-0.474**	0.517**	0.470**	0.190**	-0.272**	0.800**	-0.438**	0.791**
PIGLOG 105											
SL1 (3)		0.708**	0.031ns	-0.882**	0.724**	0.743**	-0.128ns	-0.661**	0.069ns	-0.584**	0.141*
SL2 (4)			-0.153*	-0.935**	0.754**	0.820**	-0.256**	-0.760**	-0.046ns	-0.577**	0.125ns
ML (5)				0.204**	0.046ns	-0.070ns	0.447**	0.214**	0.422**	-0.056ns	0.268**
Mesnatost, % (6)					-0.778**	-0.841**	0.270**	0.787**	0.035ns	0.605**	-0.129ns
DISSECTION											
SL1 (7)						0.812**	-0.197**	-0.768**	0.020ns	-0.677**	0.108ns
SL2 (8)							-0.284**	-0.772**	-0.035ns	-0.628**	0.074ns
ML (9)								0.502**	0.495**	0.108ns	0.266**
Meatiness, % (10)									0.352**	0.590**	0.079ns
Meatiness, kg(11)										-0.055ns	0.819**
JUS											
Meatiness, % (12)											0.148*

Conclusion

It can be concluded that the PIGLOG 105 established the backfat thickness, depth ML and the percentage of meat in live animals with a lower degree of accuracy in relation to the dissection of animals. It should be noted that the accuracy of measurements of back fat thickness and ML depth by ultrasound was affected by the following factors: body mass at the end of the test, the number of the fascia on the back fat, the movements of the animals during the measurement, the probe of the apparatus (size and frequency at which the probe runs) mistakes made by ultrasonic apparatus in measurements, as well as systematic errors made by the operators themselves in measurements. Also, it can be concluded that JUS with a lesser degree of reliability assesses meatiness/lean meat content of carcass sides compared to PIGLOG 105. All this confirms the fact that the Yugoslav standard for fattening pigs for industrial processing JUS E.CL.021 adopted in 1969 and modified in 1985 does not give a sufficiently accurate estimation of meatiness/lean meat content at the slaughter line. From the above, we conclude that it is necessary to edit the old or adopt the new Regulation for the estimation of meatiness at the slaughter line, as soon as possible.

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Pouzdanost procene mesnatosti piglog-om i povezanost osobina kvaliteta trupa

Č. Radović, M. Petrović, N. Brkić, N. Parunović, D. Radojković, I. Radović, R. Savić, M. Gogić

Rezime

Cilj rad je bio da se utvrdi pouzdanosti merenja PIGLOG-om 105 debljine slanine (SL1 i SL2), dubine *Musculus longissimus-a* (ML-a) i procene mesnatosti na živim i zaklanim grlima (disekcijom i na liniji klanja pomoću važećeg Pravilnika R. Srbije). Istraživanje je sprovedeno na 170 grla dva genotipa švedski landras (n=73) i F_1meleza ŠLxVJ (n=97), oba pola (muška kastrirana grla n = 79 i ženska grla n = 91). Pri prosečnoj telesnoj masi od 109.6 kg utvrđena je prosečne

debljine slanine PIGLOG-om 105 iznose SL1=14.55 mm, SL2=12.74 mm, dok je debljina slanine utvrđena disekcijom na istim mestima bila SL1=16.42 mm, SL2=14.19 mm. Dubina ML-a utvrđena PIGLOG-om 105 je 47.59 mm, dok je utvrđena veća dubina ML-a disekcijom (55.58 mm). Procenat i prinos mesa utvrđen PIGLOG-om 105, disekcijom i JUS-om bio je: 55.77%; 57.72% odnosno 49.36 kg i 44.42 % odnosno 38.64 kg. Na osnovu navedenih rezultata vidimo da JUS ima apsolutne vrednosti znatno manje za udeo i prinos mesa u odnosu na PIGLOG 105 i disekciju. Utvrđeno je da postoji statistički visoko značajna razlika ($P<0.01$) između procenjenih mesnatosti. Povezanost između osobina merenih PIGLOG-om 105 i istih utvrđivanih na polutkama disekcijom vidi se da je korelacija za SL1 jaka ($r=0.724$), za SL2 vrlo jaka ($r=0.820$), dok je za dubinu ML-a srednja ($r=0.447$). Korelacija za procenat mesa je vrlo jaka ($r=0.787$). Između procenjene mesnatosti pomoću PIGLOG-a 105 i disekcijom korelacije su bile statistički visoko značajne ($P<0.01$).

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INTERVENTIONS TO PREVENT INTRAUTERINE GROWTH RESTRICTION

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Abstract: Intra uterine growth restriction (IUGR), as an impaired growth of the foetus or its organs, occur naturally in pigs and results in low birth weight, which causes economical losses in pig production. This paper aims to present a hypothesis how arginine, a functional amino acid, can at least partly prevent IUGR in pigs. As a conclusion, it can be hypothesized that maternal dietary inclusion of arginine may increase blood flow and thereby nutrients to the foetus, which may result in higher birth weight, muscle fibre number and increased survival rate and postnatal growth.

Key words: Intra uterine growth restriction (IUGR), pig, arginine

Introduction

Intra uterine growth restriction (IUGR) is defined as an impaired growth of the foetus or its organs during foetal development and growth. IUGR occur naturally in pigs and results in low birth weight and contributes to a large variation in birth weight. The reason for IUGR is caused by undernutrition due to placental insufficiency *(Wu et al., 2006)*. For two reasons, IUGR causes economical losses in pig production: 1) low birth weight piglets have a lower survival rate and 2) those low birth weight piglets that survive have a decreased performance. In humans nutrients restriction can cause permanent changes in physiology later in life *(Barker et al., 1998)*. Further, several meat quality traits are dependent on the metabolic potential of the pigs at slaughter. The present short communication aims to present a hypothesis how arginine, a functional amino acid, can at least partly prevent IUGR in pigs.

Survival of low birth weight piglets

In the last few decades, selectin for increased litter size has been carried out and in Denmark and litter size has increased from 13.3 piglets per litter in year 2000 to 16.3 piglets per litter in 2010 and this has consequences on survival rate. Thus, increasing litter size decreases mean birth weight and the percentage of piglets with a birth weight lesser than 1 kg. Because the survival rate is decreased in low birth weight piglets compared with high birth weight piglets selection increases the percentage of piglets born dead. Further, the low birth weight piglets surviving have a lower performance.

Performance of low birth weight piglets

We have recently reviewed the effect of birth weight on performance *(Nissen and Oksbjerg, 2009; Oksbjerg et al., 2013)*, and in addition we published our recent results on performance traits of low birth weight pigs compared to high birth weight piglets *(Nissen and Oksbjerg, 2011)*. The general finding was that the daily gain was reduced by almost 100 gram per day during the lactation period and 87 gram per day from weaning to slaughter. This was due to a reduction in feed uptake and a tendency for an increased kg feed per kg gain *(Nissen and Oksbjerg, 2011)*. The reason for this may be due to a lower muscle fibre number. The latter being positively related to muscle growth and meat percentage, negatively related with kg feed per kg gain). Also increases in muscle fibre cross-sectional area in low birth weight piglet at slaughter has been report ed compared with high birth weight pigs. Results on meat quality traits are not consisting. However, some studies have shown increased shear force of meat from low birth weight pigs compared to high birth weight pigs and recently we found and interaction between maternal dietary protein intake and gender. Thus, in male pigs from sows feed a low protein diet, the shear force was reduced compared to male pigs born to sows fed adequate protein, suggesting that changes in the environment in the uterus may programme meat quality development in intact male pigs.

Interventions to prevent IUGR

If it is possible to increase birth weight of low birth weight piglets this may increase the survival and postnatal performance and thereby decrease the economic loss. Nutrient uptake across the placenta follows Fick's principle saying:

Nutrient uptake = (A-V) x blood flow

Where (A-V) is the arteriovenous difference, suggesting that nutrient uptake can be increased by increasing the arteriovenous difference or by increasing the blood flow.

Thus, suggested means to increase the arteriovenous difference encompass increased global maternal nutrition, level of dietary maternal protein nutrition, treatment with porcine Growth Hormone (pGH) and dietary maternal carnitine on birth weight.

Neither increased maternal global nutrition nor increased maternal protein affect the birth weight, postnaal growth or muscle fibre number of the offspring *(Nissen et al., 2003)*. On the other hand, L-carnitine fed to the piglets during the lactation period may increase the number of muscle fibres in low birth weight piglets but not in high birth weight piglets *(Lösel et al., 2009)*. Thus, further experiments are needed on the effect of maternal dietary carnitine on birth weight. pGH may redirect nutrients to the foetus, and it has been reported that injection of pGH in early windows of gestation increases the number of muscle fibres. However, the use of pGH in pig production is not approved.

Arginine is a functional amino acid. Besides being used for protein syntheses and as an intermediare in the urea cycle it is also a precursor of nitic oxide (NO) and polyamines. Nitric oxide is a potent vaso-dilator in the regulation of blood flow and polyamines support the proliferation and differentiation of cells *(Wu et al., 2006)*.

Consequently, it can be hypothesized maternal dietary inclusion of arginine may increase blood flow and thereby nutrients to the foetus, which may result in higher birth weight, muscle fibre number and increased survival rate and postnatal growth

At present we are testing the above hypothesis and results show that arginine added to the maternal diet increase daily gain of the offspring *(Krogh et al., 2016, Accepted for publication)*.

Intervencije u sprečavanju ograničenja IUGR

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Rezime

Restrikcija rasta unutar materice - Intra uterine growth restriction (IUGR), kao poremećaj rasta fetusa ili njegovih organa, se javlja prirodno u svinja i rezultira u manjim težinama na rođenju, što dovodi do ekonomskih gubitaka u proizvodnji svinja. Ovaj rad ima za cilj da predstavi hipotezu kako arginin, funkcionalna amino

kiselina, može barem delimično da spreči IUGR kod svinja. Kao zaključak, može se pretpostaviti da majčina ishrana uz uključivanje arginina može povećati protok krvi i samim tim i protok hranljivih materija do fetusa, što može dovesti do većih težina na rođenju, broja mišićnih vlakana i povećanja stope preživljavanja i postnatalnog rasta.

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THE IBERIAN PIG BREED: POPULATION, PRODUCTION SYSTEMS AND BREEDING PROGRAMS

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Abstract: The *Iberian* pig is an autochthonous breed widely extended in the Iberian Peninsula, with some racial peculiarities and different denomination (*Alentejano*) in Portugal. Since centuries, the breed has been subject to an adaptation processes to the severe conditions of a semiarid continental climate profiting of the natural resources of the sparse Mediterranean woodlands, named *dehesa*. Its traditional extensive production system is focused to obtain dry products of high organoleptic quality, mainly hams, forelegs and loins.This ancestral production system based on Iberian pigs fattened in the *dehesa* ecosystem, has been replaced mostly in the last 20-25 years by management and feeding systems related with the usual intensive pig production, based on cross breeding with the Duroc breed. The geographical area where the Iberian pig production was dominant has spread to other regions, and the cycles of balance between supply and demand lead to a huge productiveand economic crises not yet finished. Currently, they coexists several systems according to genetic type and feeding, with different market segments. Breeding program experiences are today very scarce. The official selection program involvesvery few breeders and it applies an intra-breed index because the lack of genetic connection between farms. There are some other initiatives, public and private, but the response to selection is stillvery limited.The aim of this paper is to provide some information about the Iberian pig breed, history and population, production systems and the breeding program including some perspectives about the application of new methodologies.

Key words: Iberian breed, extensive systems, cured products, quality traits

History and population

An important extension of the territory of the south-west of the Iberian Peninsula is occupied by the Mediterranean woodlands in which evergreen holm and cork oaks predominate. In most part of this woodland, shrub growth has been reduced by man in order to obtain large areas of grassland between the oaks (*dehesas*) well suited for livestock: cattle, sheep and pigs. The acorns mature in

autumn and they can be consumed by pigs, besides pastures, along all the winter. The energetic diet based in the acorns become in very fatty pigs whosemain cuts could be salted and cured and provide people long-term sustenance in an environment of scarce resources. The *dehesa* ecosystem constitutes an interesting ecological model of interaction among woodland, grassland and livestock and an important reserve for wild fauna and flora.

The characteristics of the Iberian pig, originating from *Sus mediterraneous*, have developed according with this ecosystem, its inhabitants and its necessities *(Silió, 2000)*. The morphology makes it resistant to sunstroke and high summer temperatures and enables it to travel far in search for food: dark skin and hair colour, a pointed snout and legs that are both long and strong. It can endure long periods of hunger because of its low basal metabolism and the early formation of fatty tissues. The thick layer of subcutaneous fat and the high level of intramuscular fat make its meat adequate for dry-cured meat processing.

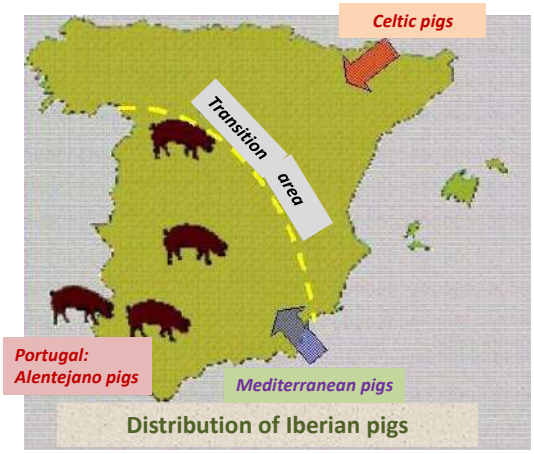

Figure 1. Distribution of Iberian pigs in the Peninsula

The extensive area of *dehesa* (Figure 1) and the absence of a selective preponderance of a standard racial or any group of breeders, allowed multiple varieties, relatively isolated from one another, which still were present in the *dehesas* in the 60-70 years of the last century. But the African swine fever (ASF) and the indiscriminate crossbreeding with foreign breeds (mainly Duroc but also Large-Black or Tamworth), reduced the population to near extinction in the following years. Moreover, the new nutritional tendenciesagainst fatty diets and the null conservation policyof the *dehesa*,increased the population reduction.

From mid 90s the situation was radically opposed: The ASF control and the economic improvement of the Spanish people causes an extension of the demand of high quality foods, especially thoseproduced in sustainable systems from an environmental point of view. The high price of live animals and products

led to the increase of Iberian pig census even outside of the traditional breeding area, with proliferation of intensive production farms.The increased supply far exceeded the demand and this situation, linked to the general economic crisis that began in 2008,brought another great population reduction and the depreciation of the Iberian pigproducts. Between 2008 and 2014 the decrease in slaughtered animals was 43%. At present, census is recovering again to a level similar to the years before the crisis (Figure 2).

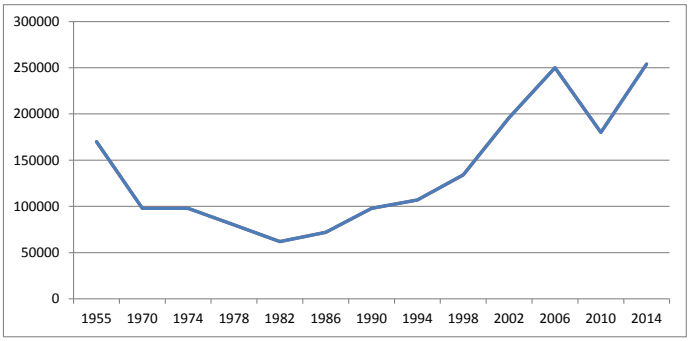

Figure 2. Evolution of the number of Iberian sows (1955-2014)

Production systems

As it has pointed before, crossbred Durocx Iberian pigs are the main genetic type produced. TheOfficialProductionRule of Iberian pig meat and cured products forced the use of purebred Iberian sows, so Duroc can only be introduced by the sire line. Crossbreeding was massive in the intensive production system but in the last years has also been preferred by producers in the traditional extensive production system, named *montanera*. Table 1 presents the official percentage of pigs marketedin year 2014 bygenetic type and production systems (total of 2.381.080 carcasses marketed) and Figure 3 shows a schematic representation of fattening categories.

Table 1. Percentages of Iberian pigs marketed in 2014 (from *ASICI, 2014*)

	Montanera[1]	*Campo*[2]	Intensive	Total
Purebred Iberian	5.1	0.9	0.6	6.7
Crossbred Duroc	12.6	12.1	68.6	93.3
Total	17.7	13.0	69.2	100

1. Acorn and pastures feeding: *bellota*and *recebo*
2. Free range without acorn and with compound feeds

About a 93% of the slaughtered pigs were crossbreds and nearly 70% were fattened in an intensive production system. The official statistic quantifies in 5%

the traditional *montanera* extensive system carried out with pure Iberian pigs. Crossbreeding with Duroc provides higher daily gain and lean percentage (*Dobao et al., 1987; Perez, 2008; Sánchez, 2011*) and it is demandedby most of the dry-product industries. There is not intensive production with purebred Iberian pigs (Table 1). In the intensive system, the pigs reach the slaughter weight (150-160 kg) at 9-10 months of age. The management and feeding follow the usual rulesfor producing heavy pigs, with some differences in the composition of the compound feeds to obtain a more unsaturated fatty acid profile in subcutaneous fat.

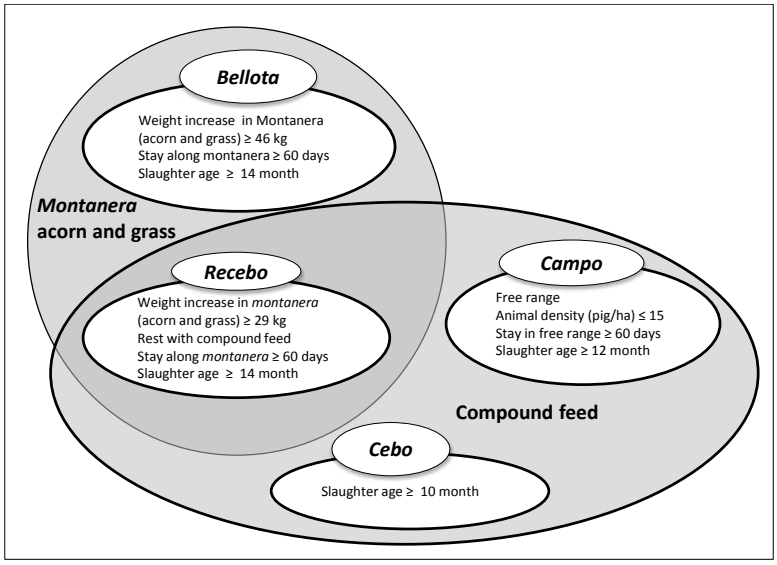

Figure 3. Schematic representation of the feeding categories included in the 2007 Quality Standard legislation (*García Casco et al., 2013*)

However, the world prestige of Iberian dry-cured products comes from pigs fattened on the *montanera* system, ie, that take advantage of grass and acorns in the autumn and winter. The whole production cycle is planned to dispose of pigs of 90-110 kg in early November. Until then, a common objective is the maximum use of field feeding resources: grazing on the spring pastures, stubble fields and any available crop, but supplementation with concentrate feeds is increasingly usual although with restricted feeding. In the *montanera* system, the pigs graze free in the *dehesas*, fed with acorn and variable intake of grass but as well as roots and bulbs. The body weight gain during this period is about 50-60 kg. The fattening capacity of the *dehesas* is very variable, depending on the type of land, tree density, acorn crop, autumnal rains, etc. As an average, the *dehesa* supports 0.5-1.0 pig per Ha. When the weather conditions are unfavourable, feeding in *montanera* is

supplemented with concentrate feeds in a practice named *recebo*. Finally, the free range system that makes use of the territory but with diets based on concentrate without any acorn intake is known as *campo* feeding (Figure 3).

Breeding programs

The official breeding program is managed by the Spanish Association of Iberian Pig Breeders (AECERIBER) with the financial support of the Spanish Agricultural Ministry and some Regional Institutions (*Silió, 2000; García Casco et al., 2013, 2014*). The activities began in 1992 with the participation of some traditional breeders and, to date, the selection index is exclusively intra-herd due to the lack of genetic connection between farms. In the extensive producers, AI is minority and the genealogical control of the replacement is still inefficient.

The program only considers pure Iberian production and the selection goal includes two approaches, one directed to farmers that sell piglets, at 45 kg of weight, and another to farmers that extend the cycle to heavy pigs for slaughter, always fattened in extensive systems. Consequently, this scheme of genetic evaluation combines:

- intra-herd genetic evaluation for Weight at 90 days (W90).

- genetic evaluation for growth in the fattening period and carcass composition (weights of trimmed hams, forelegs and loins) based on data annually recorded in family groups of animals sampled from some of the previous herds and tested under uniform extensive management, including fattening in *montanera* or *campo* (CC, Complete Cycle)

Reproductive traits like litter size has been included in the last version of the program approved by the Ministry, but the scarce genealogical information available is not sufficient to accurately estimate the breeding values of low heritable traits such as prolificacy. On the other hand, the production costs of piglets represent a very little proportion of the production costs of heavy pigs.

Single-trait (for W90) or multi-trait (for CC traits) BLUP-animal models, with random and fixed effects, are adjusted to the data, and the breeding values are weighted by the economic value in the aggregate genotype. The intramuscular fat measured in loin in most of the animals, as a quality trait, is also evaluated but is not included in the aggregate.

Private Breeding Programs. In the last years some breeders have shown interest in developing their own breeding programs, appropriate to their productive peculiarities. The selection activities handled by several multinational breeding enterprises are not well known, but there are other programs developed by Spanish

organizations specifically designed both to traditional free-range and intensive production systems.

The use of IA and the rigorous genealogical control allow including prolificacy and maternal ability in the breeding goals of all the schemes. However, the growth traits are treated in a different way in free-range and intensive systems. The official regulation forces an age at slaughter not lower than10 months for pigs fattened in intensive system and not lower than 14 months for those fattened in *montanera*. Carcass performance selection is focused to obtain high yield for premium cuts but there is great emphasis in the preservation of quality characteristics. Measures of intramuscular fat content or fatty acids profile must be taken into account to avoid negative consequences of selection for lean percentage.

New methodologies

The development of some research projects and private breeding programs has enabled availability of phenotypic records in quality traits expensive to be measured (intramuscular fat, fatty acids profile, tenderness and color of meat, drip loss). Besides the opportunity to characterize the breed that such information suppose, in the last years several studies about the genetic background of those traits has been performed (*Fernández et al., 2008; Alves et al, 2013; García Casco et al., 2015*). Some single nucleotide polymorphisms in the genes *CAST*, *LEPR*, *IGF2*, *FABP*, etc have been associated to productive and quality traits in purebred and crossbred pigs. They may provide a future a promising tool for genetic improvement. Genotyping for a concrete polymorphism of candidates to selection, particularly candidate sires, could be a possible strategy in coming years.

Iberijska rasa svinja: populacija, proizvodni sistemi i odgajivački programi

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Rezime

Iberijska svinja je autohtona rasa široko prostranjena na Pirinejskom poluostrvu, sa nekim specifičnostima rase i različitim nazivima (*Alentejano*) u Portugalu. Vekovimaa, ova rasa se prilagođavala na teške uslove polusušne kontinentalne klime, koja je profitirala od prirodnih resursa retkih mediteranskih šuma, po imenu *dehesa*. Tradicionalni ekstenzivni sistem proizvodnje je usmeren na dobijanje suvomesnatih proizvoda visokog organoleptičkog kvaliteta, uglavnom šunke, kolenica i slabine. Ovaj proizvodni sistem predaka zasnovan na iberijskoj

svinji tovljenoj u *Dehesa* ekosistemu, je uglavnom zamenjen u poslednjih 20-25 godina sistemom menadžmenta i ishrane koji se primenjuje u intenzivnoj proizvodnji svinja, na osnovu ukrštanja sa rasom durok. Geografska oblast u kojoj je proizvodnja iberijske svinja bila dominantna se proširila i na druge regione, a ciklusi ravnoteže između ponude i tražnje koji dovode do velikih proizvodnih i ekonomskih kriza, još nisu završen. Trenutno, oni koegzistiraju sa nekoliko sistema u skladu sa genetskim tipom i ishranom, sa različitim segmentima tržišta. Iskustva u programu oplemenjivanja su vrlo retka. Zvanični odgajivački program uključuje vrlo malo odgajivača i uključuje indeks unutar rase zbog nedostatak genetske povezanosti između farmi. Postoje neke druge inicijative, javne i privatne, ali je reakcija na selekciju i dalje veoma ograničena. Cilj ovog rada je da pruži neke informacije o iberijskoj rasi svinja, istoriji i populaciji, proizvodnim sistemima i programu uzgoja, uključujući neke perspektive u primeni novih metodologija.

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CONNECTEDNESS BETWEEN MANAGEMENT UNITS AND RELIABILITY OF ACROSS HERD EVALUATION IN LANDRACE AND LARGE WHITE PIGS

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Invited paper

Abstract: Impact of connectedness between management units on reliability of genetic evaluation was analyzed for Landrace (L) and Large White (LW) pigs and their crossbreds on 41 859 data records from three large scale pig farms and 70 family farms in period 1998-2014. Connectedness rating (CR) method was used to assess information about the level of connectedness. Average reliability of estimated breeding values was calculated for all contemporary groups in single and joint analysis for different combination of data. Focal trait for the analysis was backfat thickness. Average CR was less than 0.03 between all pairs of contemporary groups. Thus, across-herd validation would be unreliable due to low connectedness. After inclusion of data of crossbred animals in the data set, reliability increased from 0.24 to 0.38 on farm B in L and from 0.03 to 0.31 on farm C for LW. Results illustrate necessity to increase connectedness in order to increase individual of genetic evaluation and reliability of across-herd comparison. Adequate tool for increasing connectedness would be well organized artificial insemination.

Key words: Pigs, connectedness, reliability, genetic evaluation

Introduction

Pedigree based models have been widely used for genetic evaluation of economically important traits in pigs *(Henderson, 1975)*. However, if there are few or no genetic links between contemporary groups in the genetic evaluation, comparisons of breeding values across contemporary groups might be biased. This problem was observed in species where level of artificial insemination is low, such as beef cattle *(Veselá et al., 2007)*, sheep *(Lewis et al., 2005)* and some pig breeding programs *(Sun et al., 2009)*. *Kennedy and Trus (1993)* suggested that the most appropriate measure of connectedness would be the average prediction

variance of differences in estimated breeding values between animals in different contemporary groups. *Mathur et al. (1998)* proposed connectedness rating method, expressed as the correlation between the estimates of contemporary groups effects. Reliability of estimated breeding values is highly correlated with the level of connectedness between management units. Prediction error variance of predicted breeding values is increased in across-herd evaluation if the level of connectedness is low. With the increase of connectedness, prediction error variance is decreased and reliability of predicted breeding values is expected to be increased *(Kennedy and Trus, 1993; Lewis et al., 2005).*

The aim of this study was to determine the level of connectedness between contemporary groups within the breeding program and reliability of the genetic evaluation using different data sets.

Material and methods

Data set for productive traits contained 41,859 data records for gilts and young boars collected in field test from 1998 to 2014. Descriptive statistics for analysed trait (Table 1) were obtained by SAS statistical package. Following genotypes were included in the sudy: Landrace (L), Large White (LW) and their reciprocal crossbreds.

Table 1. Number of animals by genotype and large scale (A-C) and a group of family farms (F)

Farm	N	Mean (BF, mm)	SD	L	LW	♀ LWxL ♂	♀ LxLW ♂
A	18,176	11.89	2.36	5,136	4,667	4,196	4,177
B	6,738	10.91	1.90	2,992	973	2,738	35/
C	3,343	11.38	2.47	2,789	137	417	/
F	13,602	10.51	2.14	8,362	4,217	662	361
Total	41,859	11.10	2.33	19,279	9,994	8,013	4573

L – Landrace; LW-Large White

Connectedness rating method (CR), proposed by *Mathur et al.* (1998) was used to assess level of connectedness between management units within breeding programme. The single trait model for backfat thickness was used to assess connectedness rating for the purpose of this study. Data were analyzed with the linear mixed model:

$$\mathbf{y} = \mathbf{Xb} + \mathbf{Z}_l\mathbf{l} + \mathbf{Z}_a\mathbf{a} + \mathbf{e}$$

where **y** is a vector of phenotype observations for backfat thickness, **b** is a vector of unknown parameters for fixed effects (sex, herd-year-season effect, where season was defined as year-month of testing, genotype, and weight at the end of test), **l** and **a** are the vectors of unknown parameters for the random effect of common litter environment and breeding value, respectively; **e** is a vector of residuals, while **X**, $\mathbf{Z}_l$, and $\mathbf{Z}_a$ are design matrices linking phenotype records with corresponding parameters. The equation system was solved using PEST program (*Groeneveld et al*., 1990) in order to obtain estimated breeding values and prediction error variances (PEV). The CR between herds *i* and *j* is defined as a correlation between estimates $\hat{h}_i$ and $\hat{h}_j$ for herd *i* and herd *j*, respectively:

$$CR = \frac{\mathrm{cov}\left(\hat{h}_i, \hat{h}_j\right)}{\sqrt{\mathrm{var}\left(\hat{h}_i\right)\mathrm{var}\left(\hat{h}_j\right)}}$$

The average CR between one management unit and all other management units can be defined as the average of CR with all other herds in analysis. Average CR was calculated between herds in the last year in the dataset. The calculations were performed for different combinations of data in the sets. In the first case, CR between contemporary groups was calculated using purebred data separately for L and LW. In addition, CR was calculated using data for both pure breeds and their crossbreeds. Reliability of estimated breeding values (r^2) was calculated as:

$r^2 = 1 - (PEV / \sigma^2_a)$,

where PEV is prediction error variance and σ^2_a is additive genetic variance in population *(e.g. Mrode, 2005)*.

Results and discussion

Average CR within breeding programme for L was lower than 0.03 between all management units (Table 2), which is recommended minimum level for across herd comparison of breeding values when calculation is based on backfat thickness data *(Mathur et al., 2002)*.

Table 2. Connectedness rating between management units for Landrace

Farm	A	C	D	F
A	0.019	0.002	0.001	0.001
B	/	0.020	0.003	0.002
C	/	/	0.020	0.003
F	/	/	/	0.029

A-C – large scale farms; F- group of family farms

However, CR within the group of family farms was very close to desired 3% and with additional efforts for increasing connectedness across herd comparison within this group might be conducted.

Table 3. Connectedness rating between management units for Large White

Farm	A	C	D	F
A	0.056	0.020	0.025	0.025
B	/	0.023	0.002	0.002
C	/	/	0.020	0.020
F	/	/	/	0.029

A-C – large scale farms; F- group of family farms

Similar situation was observed in LW (Table 3), although CR between farm A and family farms was 0.025, which is close to the desired value of 3% and can be used as good starting point for across herd comparison of evaluated animals. No substantial change can be observed when crossbred data are included in data set (Table 4). This is probable result of crossbreeding only within farms, where no exchange of animals for crossbreeding between farms was done and consequently, no increase in connectedness was noticed.

Table 4. Connectedness rating between management units with purebred and crossbred data included

Farm	A	C	D	F
A	0.066	0.020	0.016	0.015
B	/	0.024	0.009	0.004
C	/	/	0.020	0.004
F	/	/	/	0.130

A-C – large scale farms; F- group of family farms

Mathur et al. (2002) found that average CR between management units within Canadian pig breeding programme was below 3%. However, the level of connectedness increased over defined limit for joint evaluation of animals when common sires were used and spread over entire population. *Sun et al.* (2008) found

that average CR within L and LW in China was generally low, implying that joint genetic evaluation could be done only for few herds.

Despite the low level of connectedness, slight increase in reliability of estimated breeding values was noticed within management units with large number of data records when additional amount of data was included in analysis (Table 5 and Table 6). Remarkable increase in reliability was noticed in contemporary groups with smaller number of observations. The difference in average reliabilities for the farm B was from 0.24 to 0.38 in L and from 0.03 to 0.31 on farm C for LW. The increase in reliability of estimated breeding values in all management units is due to enlarged amount of information when joint evaluation was done.

Table 5. Average reliability of prediction within contemporary groups using purebred data

Farm	**Data set (DS)**			
	DS1		**DS2**	
	L	**LW**	**L**	**LW**
A	0.32	0.33	0.37	0.36
B	0.24	0.22	0.27	0.28
C	0.28	0.03	0.29	0.07
F	0.26	0.05	0.29	0.06

DS1 – reliability of genetic evaluations within breeds and management units;
DS2– reliability of genetic evaluations for purebreds and crossbreds within management units;

Similar results were found by *Lutayaa et al.* (2002) and *Ibañez-Esriche et al.* (2011), who also found increase in reliability when additional data were included in evaluation. Although values of CR between contemporary groups were low, increase in reliability implies that genetic evaluation should be more reliable if across-herd comparison is done. By increasing the level of connectedness, additional improvement in reliability of breeding values can be expected, as suggested by *Lewis et al.* (2005).

Table 6. Average reliability of prediction in contemporary groups using purebred and crossbred data

	Data set (DS)			
Farm	**DS3**		**DS4**	
	L	LW	L	LW
A	0.38	0.38	0.43	0.42
B	0.32	0.27	0.38	0.38
C	0.34	0.09	0.35	0.31
F	0.31	0.13	0.33	0.21

DS3 – reliability of across-herd genetic evaluations within breed;
DS4 – reliability of across-herd genetic evaluations for purebreds and crossbreds;

Connectedness among management units contribute to reliability of breeding values when across-herd comparison is done. However, the level of connectedness depends on the exchange of genetic material between farms, but also on the frequency of using and organization of artificial insemination within breeding programmes. Organization of the artificial insemination plays an important role in increasing the level of connectedness between management units.

Conclusions

Due to insufficient genetic links between herds results of genetic evaluations should be compared only within herds. The increased amount of information about individual, but also on its relatives, increases reliability of the prediction. Use of common sires should be used as it is the most efficient tool for increasing the level of connectedness between contemporary groups and consequently reliability of prediction within particular breeding programme.

Povezanost između upravljačkih jedinica i pouzdanosti evaluacije zapata landras i jorkšir svinja

Z. Luković, D. Škorput

Rezime

U ovoj studiji smo analizirali povezanost unutar upravljačkih jedinica (velikih farmi i porodičnih farmi) uključenih u hrvatski nacionalni program uzgoja svinja i pouzdanosti genetskog vrednovanja u zapatu svinja rase landras i jorkšir i njihovih meleza. Povezanost je analizirana metodom ocene izražena kao korelacija ocena $\hat{h}_i$ i $\hat{h}_j$ za zapate *i* i *j* koja je zasnovana na proceni vrednosti uzgoja za debljinu leđne slanine. Prosečna pouzdanost procenjenih priplodnih vrednosti je izračunata za sve savremene grupe u jednoj i zajedničkoj analizi za različite kombinacije podataka. Utvrdili smo nizak nivo povezanosti između svih upravljačkih jedinica. Zbog niskog nivoa povezanosti, evaluacija čitavog zapata se ne preporučuje zbog povećanja varijanse greške predviđanja kao posledice nedovoljnih genetskih veza između upravljačkih jedinica. Nema značajne promene u povezanosti kada su u skup podataka uključeni podaci o melezima. Ovo je verovatno posledica ukrštanja samo unutar farme, gde nema razmene životinja za ukrštanje između farmi i samim tim, nije primećen porast povezanosti. Povećanje varijanse greške predviđanja će smanjiti pouzdanost genetskog vrednovanja svinja. Priplodne/odgajivačke

vrednosti iz seta podataka sa velikim brojem fenotipskih podataka su imali veću pouzdanost zbog uvećane količine informacija i uzimajući u obzir genetske veza između zapata. Najkorisnije sredstvo za postizanje većeg stepena povezanosti između zapata je upotreba zajedničkih svinja iz centara za veštačku oplodnju i adekvatne organizacije veštačke oplodnje.

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NUTRITIONAL CHALLENGES IN ENTIRE MALE PRODUCTION

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Abstract: When feeding entire males, one is facing two major challenges. Firstly, the diet needs to cover the nutrient requirements for the greater muscle protein deposition capacity of uncastrated compared to castrated and female pigs. Secondly, there is clear evidence that with specific dietary ingredients the production of skatole and indole, two of the three components known to be responsible for the development of boar taint in the hindgut, can be modulated. This short non exhaustive review will summarize some of the knowledge acquired over the last years and discuss new challenges European pig production will face if castration of entire male pigs will be prohibited in the near future.

Keywords: nutrient requirement, feeding strategy, boar taint, gut, entire male

Nutrient requirements of entire males

Whole body nutrient composition – new data show increased efficiency

Preliminary results of a sequential slaughter study conducted in Switzerland show that as a result of a targeted breeding strategy to obtain leaner carcasses, empty body protein content increased in the last 30 years on average by 20% from 22 to 26 g/kg *(Stoll and Ruiz, 2015)*. Interestingly, under optimal husbandry conditions as it was the case in the experiment of *Stoll and Ruiz (2015)*, nitrogen utilization efficiency of almost 50% could be achieved. Compared to barrows and gilts, the empty body protein content of entire males reached even 28 g/kg above 70 kg live weight. Especially the time point when differences in lean tissue deposition is getting evident coincides with findings obtained on a longitudinal DXA measurement study of *Suster et al. (2006)* where they compared lean and fat tissue deposition rate ofentire males and barrows between the age of10 to 150 d. They reported that only in the late finisher period daily lean tissue deposition rate of entire males compared to barrows was significantly greater

ranging from 22 to 58 g/d. They did not report any improvement in growth rate but due to the markedly lower propensity to deposit fat, as in other studies *(Pauly et al., 2009; Quiniou et al., 2010)*, better feed conversion of group penned boars were observed.

How different are the amino acid and protein requirements of entire males?

There are very few and sometimes contradictory data published in the literature comparing the protein and lysine requirements of entire males and barrows *(Dunshea et al., 2013)* whereas more published data comparing entire males and gilts are available *(Batterham et al., 1985; Giles et al., 1986; King et al., 2000; O'Connell et al., 2005, 2006)*. However, since protein and lysine requirements of gilts are similar or somewhat greater compared to barrows *(Quiniou et al., 2010)*, the values for the female pigs can serve as reference. Keeping that in mind, using data of 13 published studies *Dunshea et al. (2013)* estimate dietary lysine requirements for boars in the finisher period of 0.6 g SID/MJ DE, which is 9% greater compared to gilts. Given the fact that the slope of the protein dose response curve derived from the meta-analysis study was greater in entire males compared to gilts implied a greater impact of inadequate dietary lysine supply on growth performance. The simulation study with the InraPorc software performed by *Quiniou et al. (2010)* not only confirmed the greater lysine requirement of entire males compared to gilts and barrows but also suggested that the decline in lysine requirement over time was markedly slower in entire males compared to female and castrated pigs. In good agreement with the simulation study, *Ruiz et al. (2015)* showed that entire males reacted more severely to a protein, lysine, methionine + cysteine, threonine and tryptophan reduced diet [20% below Swiss feeding recommendations for Swine, *(Agroscope, 2015)*] compared to gilts and barrows. In this study together with total dietary protein content only four essential amino acids were taken into account for feed formulation. Consequently, other essential amino acids such as isoleucine were at 70% of recommended dietary level. Despite the unbalanced diet, empty body nutrient composition of female pigs and barrows but not of entire males in the low and standard protein group was similar. Interestingly, studies exist which suggest that for maximizing protein deposition and feed efficiency no additional lysine is required for entire males compared to gilts *(King et al., 2000; O'Connell et al., 2005)*. One possible explanation for the discrepancy between studies could be the rearing conditions. The aforementioned studies were carried out with individually penned pigs. When animal-animal interactions, especially those related to sexual behaviour, are prevented, potential for maximal feed intake is guaranteed *(Pauly et al., 2009)*. In

such “experimental” conditions feed intake of entire males can be similar or even greater than of gilts *(King et al., 2004)*.

Immuno castration and its impact on amino acid and protein requirements

As discussed in the review of *Dunshea et al. (2013)*, protein deposition rate of immune castrates, an other alternative to surgical castration, remains for four weeks after the second vaccination as high as for entire males. Therefore, similar protein and lysine requirements can be foreseen for that time span. Nonetheless, one needs to account for the fact that within a short period of time after the second vaccination, feed intake markedly increases *(Pauly et al., 2009)* and therefore lowering dietary protein and lysine content is indicated. Based on results of various studies *(Moore et al., 2011; Huber et al., 2012), Dunshea et al. (2013)* suggest to reduce lysine supply already after two to three weeks after second immunization because of the sharp decrease in amino acid requirements of these animals, which is beyond the usual decrease due to the greater body weight.

Feeding strategies against boar taint

As previously discussed the main issue in entire male production and also the main reason for commonly performing surgical castration of male pigs is the risk of tainted carcasses, which is mainly caused by the three known compounds androstenone, skatole and indole. While androstenone formation is primarily under environmental and genetic influences *(Robic et al., 2008; Zamaratskaia and Squires, 2009)*, diet composition and feeding management play a crucial role for the production and ultimately the tissue concentration of skatole and indole *(Babol et al., 1999; Zamaratskaia et al., 2005; Moerlein and Tholen, 2015)*. For the microbial synthesis of skatole in the hindgut tryptophan is needed and as postulated by *Claus et al. (1994)* this amino acid mainly originates from gut cell debris and only small amounts are of dietary origin. Feeding and feed additives can have animpact at various steps of the physiological cascade of skatole and indole synthesis *(Wesoly and Weiler, 2012)*.

Dietary effects on ileal and caecal mucosa

Experimental data show that increased mitosis and apoptosis caused by diets rich in energy and purines result in an elevation of skatole concentrations in the faeces, blood and adipose tissue *(Loesel and Claus, 2005; Loesel et al., 2006)*. By contrast butyrate-producing feed stuff such as raw potato starch show anti-apoptotic effects *(Claus et al., 2003)* resulting in low skatole formation and

deposition *(Loesel and Claus, 2005; Pauly et al., 2008)*. Because precaecal digestibility of free tryptophan is high, diets supplemented with the synthetic form of this amino acid have only a low impact on skatole formation. The impact of high vs. low crude protein content on skatole and indole production is also very weak *(Muller et al., 2014)*. By contrast diets containing animal products like blood, meat and bone meal can lead to greater skatole and indole concentrations possibly due to the greater availability of tryptophan in the hind-gut *(Leong et al., 2011)*.

Dietary effects on gut microbiome

As skatole and indole are the result of microbial degradation, dietary interventions influencing the population and/or activity of skatole- and indole-forming bacteria affect the production of the two boar taint compounds. Possible interventions include antimicrobial compounds like antibiotics and plant extracts with antimicrobial activity. Although being very radical, but only effective at high inclusion levels, the use of antibiotics for reducing skatole concentration in the adipose tissue and/or faeces is out of discussion *(Hansen and Larsen, 1994; Hansen et al., 1997)*. By contrast the use of secondary plant extracts and essential oils have the potential to affect the microbial ecosystem *(Michiels et al., 2009; Huang et al., 2012)*. To our knowledge no scientific evidence is available showing their impact on skatole formation. Recently and in good agreement with studies with small ruminants *(Girard et al., 2015)*, promising results were published which suggest that dietary components rich in hydrolysable tannins reduce skatole and indole formation in the hindgut as well as to a certain level in the adipose tissue *(Wealleans et al., 2013; Bee and Ampuero Kragten, 2015; Čandek-Potokar et al., 2015)*. Whether these effects are related to the properties of tannins to form complexes with dietary proteins or to alter the activity and/or the proportion of hindgut microbiota still needs to be assessed.

Known dietary additives, which alter the intestinal lumen pH are formic, benzoic and sorbic acid. Despite differences in their efficacy, organic acids reduces the number of coliforms, enterococci and lactic acid-producing bacteria in the gastrointestinal tract *(Øverland et al., 2007; Øverland et al., 2008)*. The same authors reported lower skatole concentrations in the plasma but not adipose tissue of entire males fed diets containing 1% formic or 0.85% benzoic acid. Similar to organic acids, easily fermentable carbohydrates are thought to alter intraluminal pH within the colon thereby affecting the microbiome. For instance, diets containing inulin, a polysaccharide of plant origin (e.g. chicory, jerusalem artichoke) significantly reduced not only skatole levels in the colon and rectum but also the enterobacteria counts in the colon and rectum *(Overland et al., 2011)*. Similarly, *Vhile et al. (2012)* reported that diets supplemented with dried Jerusalem artichoke

offered to entire males for one week before slaughter decreased skatole levels in the hindgut and adipose tissue. The authors hypothesized that the reduced skatole levels could be related to the decrease in *Clostridium perfringens* population and the concomitant lower pH in the colon due to the increased short chain fatty acid concentration.

Dietary components as energy resources for the microbiota

Besides affecting the microbial ecosystem in the gut, easily fermentable carbohydrates are thought to serve also as an energy source for the gut microbiota thereby favouring saccharolytic instead of proteolytic microbial metabolism (as reviewed by *Jensen, 2006*). Apart from inulin, the most effective supplement, which reduces skatole levels in the hindgut as well as in the fat tissue is raw potato starch included in the diet at 20% level *(Claus et al., 2003; Chen et al., 2007; Pauly et al., 2008; Overland et al., 2011)*.

Alternative nutritional approaches to control boar taint

As previously mentioned, up to now there has not been reports on dietary manipulations targeting the concentration of androstenone. However, recently *Jen and Squires (2011)* reported that androstenone level could be lowered in the blood and fat tissue by using non-nutritive sorbent like active carbon. This confirmed their earlier findings obtained *in vitro* where they observed that active carbon efficiently binds androstenone as well as skatole *(Jen and Squires, 2011)*. The question arises how androstenone, which is produced by the Leydig cells of the testis, reaches the intestine. A possible explanation could be that similar to steroid hormones, androstenone undergo enterohepatic circulation *(Roberts et al., 2002)*. For oestadriol this was experimentally shown by *Ruoff and Dziuk (1994)*. The same authors observed also that treatment with oral antibiotics decreases the activity of the gut microbes, which interrupted the enterohepatic circulation by decreasing the amount of deconjugation and reabsorption of estrogens *(Ruoff and Dziuk, 1994)*. Thus, the active carbon used by *Jen and Squires (2011)*, which is extremely porous and possesses a large amount of available surface area may have absorbed androstenone thereby interrupting the enterohepatic circulation. Interestingly, hydrolysable tannins might have had a similar effect in the studies of Wealleans et al. (2013) and *Čandek-Potokar et al. (2015)*, where increasing levels of dietary hydrolysable tannins resulted in a non-significant but clear linear decrease in the levels of androstenone. Thus, one can speculate that the effect of the hydrolysable tannin was not limited to the hindgut but they might have been also active in the

small intestine where they may have affected the activity of the gut microbes in a similar way as reported by *Ruoff and Dziuk (1994)*.

Conclusion

The recent experiment of *Stoll and Ruiz (2015)* shows that with respect to the use of nutritional resources pork can be produced very efficiently with entire males. These results further revealed that the protein deposition potential of entire males from modern breeds markedly increased over the last decades. Still unclear is whether compared to the ideal protein concept the ratio among the amino acids of the whole body protein remained similar or changed as well. Given that the penalty in growth performance for having inadequate dietary amino acid is greater in entire males than in gilts or barrows, it is important to ensure that amino acid requirements are met to obtain the maximum benefits of entire male production during growth. These open questions awards further research in the future.

Various nutritional approaches have been proposed to minimize the development of boar taint. A great deal of research focused on the dietary control of skatole and indole whereas only recently strategies were proposed which aimed to control the androstenone concentration. Because all three compounds are responsible for the incidence of boar taint, in the future it might be interesting to test combination of dietary ingredients in order to assess if additive effects could be obtained and if dietary strategies exist which could constantly ensure low levels of boar taint compounds.

Izazovi u ishrani nekastriranih nerasta

G. Bee

Rezime

Kada govorimo o ishrani nekastriranih nerasta, suočavamo se sa dva velika izazova. Prvo, ishrana treba da pokrije potrebe u hranljivim materija za veću sposobnost i kapacitet deponovanja mišićnih proteina kod nekastriranih u odnosu na kastrirane neraste i nazimice. Drugo, postoje jasni dokazi da je se sa specifičnim sastojcima u ishrani, može da se utiče na stvaranje skatola i indola, dve od tri komponente odgovorne za razvoj polnog mirisa nerasta. Ovaj kratki, ali ne i konačni, pregled će sumirati neka znanja stečena tokom poslednjih godina i razmatrati neke nove izazove sa kojima će se proizvodnja svinja u Evropi suočiti ako kastracija muških svinja bude zabranjena u bliskoj budućnosti.

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AN OVERVIEW OF PROTEIN NUTRITION OF THE PURE IBERIAN PIG

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Invited paper

Abstract: An accurate assessment of the animal's requirements is of utmost importance for a balanced nutrition of farm animals. In this review we describe the results from a series of experiments, performed during the last years by our research group, aiming at assessing the utilization of dietary protein by the Iberian pig throughout the different stages of its productive cycle. Nutritional dose-response studies involving several isoenergetic treatments differing in protein concentration -all with similar aminoacid profile following the ideal protein concept- along with comparative growth and metabolic studies with conventional breeds have been performed. Our observations reveal that the capacity of this breed for protein accretion is rather limited compared to that of conventional or lean breeds at similar stages of growth. They also suggest the need for reducing the concentration of protein in the diet of the Iberian pig to comply with the metabolic profile of this native pig breed. We summarise all the information gathered during the last fifteen year sand provide recommendations on the level and composition of dietary protein in the diet for pure breed animals during the different phases of growth. An adequate nutritional management, particularly dietary protein provision, improves the efficiency of utilization of dietary protein and results in relevant economic, environmental and animal welfare benefits.

Key words: protein nutrition, Iberian pig, amino acids, growth, efficiency

Introduction

One of the metabolic singularities of the pure Iberian pig is its low genetic capacity for lean tissue deposition. The scientific evidence available shows that, in the growing pig, protein deposition is a protein-dependent process below an optimal protein supply per unit of energy. This relationship has been described as a linear function up to a breakpoint beyond which protein deposition becomes

largely dependent on energy supply (*Campbell et al., 1984, 1988; Kyriazakis and Emmans, 1992*). In Figure 1 are shown the relative proportions of protein and fat deposited during the growth and fattening of the Iberian pig. For comparison, data on Large-White pigs are also provided. Protein deposition is comparatively lower and fat deposition much higher in Iberian pigs for the body-weight ranges studied, compared with available data from conventional pigs. We measured N retention in a comparative study with Iberian and Landrace gilts of approximately 25 kg BW fed diets of 120 and 160 g crude (ideal) protein/kg (12.0 MJ EM/kg). Nitrogen retention and efficiency of N retention was significantly lower in Iberian gilts, particularly those fed the higher protein content diet (*Rivera-Ferre et al., 2006*). We have aimed also at identifying the primary causes that limit protein accretion in this native pig breed. In this sense, several years ago, we measured by isotopic techniques protein synthesis rates in different muscles and viscera of Iberian and Landrace gilts (*Rivera-Ferre et al., 2005*). Surprisingly, for the 3 muscles studied (*Longissimus dorsi, biceps femoris, semimembranosus*) synthesis rates were 25-30% higher in the Iberian pigs; however, muscles relative weights (g/kg BW) were 20-30% lower. Overall, these findings suggest that in the Iberian pig muscles both protein synthesis and degradation are comparatively higher leading to reduced protein accretion (g/d) and less body protein mass in comparison with conventional pigs. Consequently, the energy cost of protein accretion should be higher in the native breed as both processes require considerable amounts of energy. We have confirmed this in later studies (*Barea et al., 2007; Conde-Aguilera et al., 2011; Nieto et al., 2012*). Along with the higher protein turnover rates, the higher relative weights of viscera (*Rivera-Ferre et al., 2005*) makes the Iberian pig less efficient in the use of dietary protein and energy than its lean counterparts. Therefore, in the light of this metabolic profile, it seems reasonable to design a feeding system adapted to the particular metabolic needs of this native pig breed.

Materials and Methods

We have assessed protein requirement of the Iberian pig by analysing animal growth and protein-deposition responses to variable protein intakes. In this way, both the pig maximum capacity to accrete protein (Pmax, g/d) and the response in protein deposition (PD) to changes in energy intake can be determined. This last concept is called *marginal efficiency of proteindeposition* and it represents the increment in protein deposition per unit of increment in metabolizable energy intake (MEI) at restricted intakes (ΔPD/ΔMEI g/MJ). Both variables are influenced by genotype andendocrine status, so the optimal protein/energy ratio may be different for breeds with dissimilar genetic potential for PD.

Dietary protein in our experiments have been formulated always following the "ideal protein concept" (i. e., the perfect balance of essential amino acids needed for maintenance and productive functions, BSAS, 2003; NRC, 2012). All the pigs used in the experimental designs have been pure Iberian from the *Silvela* strain provided by a single producer (Sánchez Romero Carvajal Jabugo, S.A, Spain). The method used for quantifying body PD along all the experiments shown has been the comparative slaughter technique.

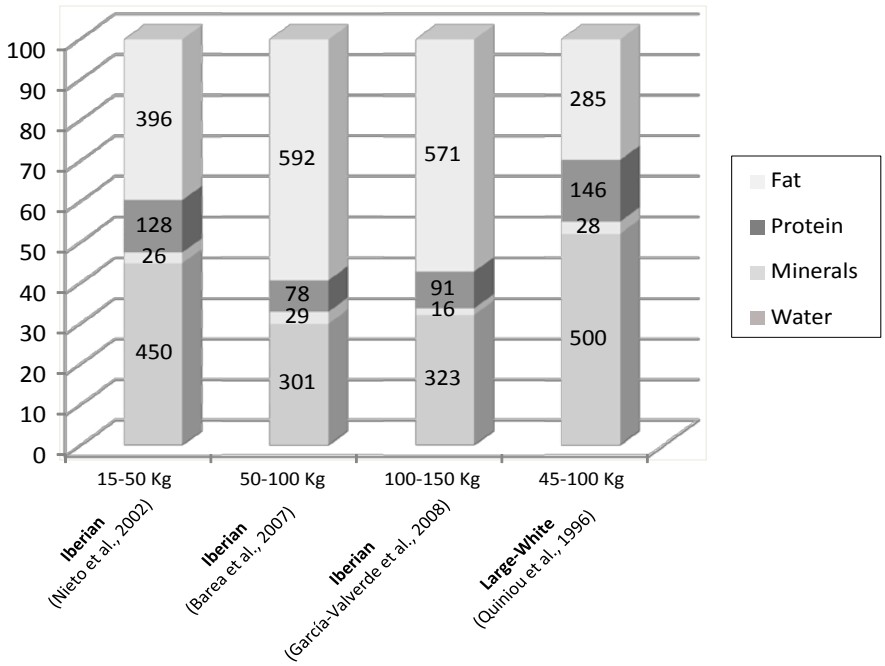

Figure 1. Body-weight gain composition of growing Iberian and conventional-type pigs.

Results and discussion

In studies with growing pigs, performed in the BW range of 15 to 50 kg BW, 6 dietary protein and 3 feeding levels were allocated into a factorial arrangement (6 × 3) with 4 pigs per treatment combination. Protein levels used were: 101, 129, 156, 175, 192 y 223 g crude protein (CP)/kg dry matter (DM), and feeding levels 0.95, 0.80 and 0.60 of *ad libitum* intake (*Nieto et al., 2002*). Experimental diets were prepared by diluting a diet of highprotein content (based on barley and soya bean meal) with a protein-free mixture base on maize starch.

Therefore,dietary protein/energy ratiowas progressively reduced without changing protein amino acid profile. Average digestible energy content was 15.2 MJ/DM. Best performance was obtained in pigs fed 129 gCP/DM diet at 0.95 × *ad libitum*, reaching 559 g average daily gain (ADG) and 74 g protein deposited/day (*Pmax*). This figure is considerably lower to those described for conventional or lean pigs which can attain values of 170 g/d or even above for this BW range. This dietary treatment provides 8.49 g CP/MJ ME, equivalent to 6.87 g digestible protein /MJ ME. The marginal efficiency of protein deposition was 2.81g/MJ.

In growing-fattening Iberian pigs (50-100 kg BW) an experiment according to a factorial arrangement (4 dietary protein × 3 feeding levels) with 6 or 7 pigs per treatment combination was carried out. Dietary treatments were obtained in a similar manner as previously described resulting in four diets containing 70, 95, 120 and145 gCP/kg DM (*Barea et al., 2007*). Feeding levels were the same as in growing pig experiments. In this case PD showed a trend to increase with decreasing dietary CP until 95 g/kg DM. With this dietary regime pigs also grew faster (854 g/d). *Pmax* reached 71g/d with pigs consuming this treatment at 0.95 × *ad libitum*. In energy terms, this diet provides6.60 g CP/MJ ME or 5.20 g digestible protein/MJ ME. The capacity for PD was considerably reduced compared with growing pigs as shown by the lower marginal efficiency of protein deposition obtained,1.43 g/MJ.

For conventional pigs of similar BW ranges, the National Research Council (*NRC, 2012*) recommends protein intakes 35 and 50% above those found to fit Iberian pig requirements. Clearly, if Iberian pig feeding programmes would follow these guidelines, the oversupply of protein would impact negatively in animal growth, lean tissue deposition, farm economy and environmental pollution.

Following similar procedures, growth, carcass and PD parameters were studied in finishing Iberian pigs (100-150kg BW, *García-Valverde et al., 2008*). For practical reasons, dietary protein level was fixed at the same value found optimal for 50-100 BW growing-fattening pigs (i. e.,95 g/kg DM) and the feeding level adjusted either at 0.95 or 0.70 × *ad libitum*, with 6 pigs allocated to each. Daily protein gain was not modified by feeding level and averaged 80 g/d (very close to previous observations in growing and fattening pigs). It is noticeable than in restrictedly fed pigs lean content/kg carcass gain was increased. Therefore, a degree of feed restriction may result in an improvement in carcass quality and deserves attention in the feeding management of the finishing Iberian pig reared indoors.

The potential for protein deposition in the pig decreases as the animal approaches maturity (*van Lunen & Cole, 1996*) and protein needs change

accordingly. For this reason, optimum protein levels during post-weaning were further investigated, as the potential for lean tissue deposition could differ from the observed in growing pigs from 15 to 50 kg BW. With this aim, an experiment to investigate protein requirement from 10 to 25kg BW was designed including 4 protein concentrations (201, 176, 149 y 123 g CP/kg DM) and 2 levels of feeding (0.95 or 0.70 × *ad libitum*) with 6 or 7 piglets per combination of treatments (*Conde-Aguilera et al., 2011*).Orthogonal polynomial contrast analysis revealed a significant linear effect of protein level upon body protein retention, suggesting that maximum PD was not achieved (no significant quadratic effect was observed). On the other hand, mean treatment comparison showed that pigs consuming the higher protein concentration diets -210 and176 gCP/kg DM-showed similar PD which suggests that the aforementioned PD values could be close to *Pmax* for this growing phase. The maximal values for ADG, and PD were obtained in piglets fed the greatest CP diet at 0.95 × ad libitum, 416 and 60 g/d, respectively. This treatment provides 11.0 g digestible protein (0.77 g digestible lysine)/MJ ME. The marginal efficiency of body PD (ΔPD:ΔME) obtained with diets supplying 210 and176 gCP/kg DM, 4.39 g/MJ of ME, indicates greater efficiency of PD when compared with previously determined values in growing and growing-fattening pigs (2.81 and 1.34 g/MJ, respectively).

For all previously described studies, dietary protein was formulated following the optimum AA pattern – in terms of g AA/kg CP – established for conventional growing pigs (*NRC, 2012; BSAS, 2003*). Nevertheless, it remained questionable if this protein profile would be the more adequate for the growth of these obese pigs. Therefore a study was designed with the aim of determining the optimum Lys proportion of dietary protein for Iberian piglets by analyzing 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, providing Lys:CP ratios of 43, 47, 52, 57, 64 and 72 g/kg. Ten piglets were allocated to each treatment. Carcass PD increased linearly and quadratically on increasing dietary Lys, reaching maximum values (39.3–40.2 g /d) with diets providing 57, 64 and 72 g Lys/kg CP. The first derivative of the quadratic function relating carcass PD and dietary Lys content gave a value of 63.7 g Lys/kg CP. Plasmaurea concentration tended to decrease on increasing Lys concentration and, both the broken- line and quadratic approach gave an estimate for Lys requirements of61.2 g Lys/kg dietary CP. However, our preferred estimate, based on carcass PD – the more reliable measure of metabolic Lysutilization – is 63.7 g Lys/kg CP, somewhat below the established for conventional piglets.

In another set of experiments was investigated the likely causes for the slow growth rate of the Iberian suckling piglet compared with lean and conventional pig types (*Aguinaga et al., 2011*). The starting hypothesis was that these lower rates of growth could be caused either by an insufficient milk nutrient supply (related to less milk intake or less nutrient concentration in the Iberian sow milk compared with leaner sows´milk) or by a decreased milk nutrient utilization efficiency. The lactation period was extended up to 34 days. Mean birth weight was 1.4 kg. The average growth rate over lactation was 168 g/d, and mean milk intake during this period 863 g/pig per day, comparable to values described for conventional piglets (*ARC, 1981*) if appropriate corrections for Iberian litter size are applied (6 piglets/litter). Moreover, the composition of Iberian sow's milk showed little differences compared to milk from other porcine breeds (*Klobasa et al., 1987*). When weight gain was related to energy intake from milk we obtained the equation: ADG (g/d) = 0.0148 milk gross energy intake (KJ/d). The slope of this equation indicates that for each MJ increment in energy intake, the growth of the Iberian suckling piglet increases by 41.8 g. In energy terms, this figure implies 24 kJ per gram of weight gain, a value 30% greater compared with similar estimations in leaner suckling piglets (18.4 MJ, *Noblet et al., 1998*). Mean values for body protein, fat and energy retention over the lactation period were 27.4 g, 22.7 g and 1615 KJ, respectively. The efficiency for protein retention was 0.59, meanwhile in lean pigs has been described as 0.85 or even higher (*Noblet & Etienne, 1986*). The conclusion from these experiments was that the lower growth rate observed in Iberian compared with conventional suckling piglets seems to be related to a lower efficiency of utilization of milk nutrients and not to a decreased nutrient intake. In further experiments with suckling piglets provided with creep feed during lactation a similar figure for whole-body PD was obtained (*Castellano et al., 2014*) suggesting that *Pmax* for this growing period is close to 27.4 g/d.

The graphic evolution of the maximum capacity of the Iberian pig for PD (*Pmax*) during growth is represented in Figure 2. According to the previously discussed results, *Pmax* increases rapidly during the earlier phases of growth (*Conde-Aguilera et al., 2011, Nieto et al., 2002*) to reach a plateau thereafter. The inflexion point corresponds to 32.5 kg BW, mid-point of the study performed with growing pigs from 15 to 50 kg BW (*Nieto et al., 2002*). Beyond this point, *Pmax* is maintained at an average value of 75 g/d. The equation that best fits this model is: $Pmax = 77.5 \pm 2.86 - 218 \pm 26.2 \times 1 / BW_{(mean)}$.

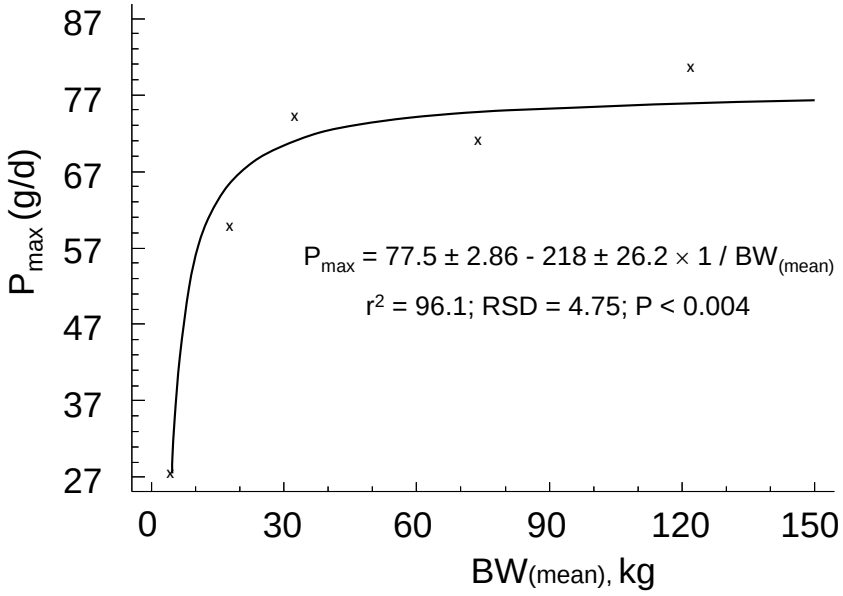

Figure 2. Evolution of maximum capacity for body protein deposition in the Iberian pig

During the previous discussion, we have already mentioned that the marginal efficiency of protein deposition, i. e., the response in PD to changes in energy intake, which is estimated as the slope of the linear relationship between PD and MEI, undergo considerable changes along the Iberian pig productive cycle. In this sense, the slope achieved a value of 4.39 g/MJ EM in post-weaned piglets from 15 to 25 kg BW (*Conde-Aguilera et al., 2011*) decreased to 2.81 g/MJ EM in growing pigs from 15 to 50 kg (*Nieto et al., 2002*), and decreased further to 1.34 g/MJ EM in growing-fattening pigs from 50 to 100 kg PV (*Barea et al., 2007*). This means that the growing phase of the pig has a determinant influence upon the effect of energy intake over the process of body protein deposition. For the last finishing period (100-150 kg BW) we have assumed that ΔPD/ΔME approaches zero, similar to what have been described in conventional pig genotypes. Overall, our results show that when the Iberian pig is fed under dietary regimes with optimum or sub-optimum protein/energy ratio, the relationship between PD and MEI decreased as the pig increases BW (age), following a curvilinear patter described by the equation:

$\Delta PD/\Delta EM = 10.6 \pm 0.46 - 2.20 \pm 0{,}117 \times \ln BW_{(mean)}$; $r^2 = 99.4$; RSD = 0.173; P < 0.003

These findings are in the line of those described for conventional pig genotypes by *Black et al. (1986)* and *Bikker (1994)*, who also observed decreases in the marginal efficiency for protein deposition as the pig increases in BW.

Finally, in Figure 3 there is a summary of the productive performance parameters observed in the Iberian pig from the post-weaning to the finishing period, along with the protein recommended intakes for each growth period. These recommendations could be useful for the nutrition of other autochthonous pig breeds whose protein requirements are completely unknown.

Body weight range	10-25 kg	25-50 kg	50-100 kg	100-150 kg
Performance parameters				
Maximum protein deposition capacity (P_{max}), g/d	60	74	71	80
Average daily gain, g/d	416	559	854	679-917
Δ protein deposition / Δ energy intake, g/MJ ME	4.39	2.81	1.43	≈ 0
Recommendations				
Crude protein (ideal) / Dry matter, g/kg	201	129	95	95
Ap. digestible protein (ideal) / ME, g/MJ	11.0	6.86	5.20	4.84
Ap. digestible lysine / ME, g/MJ	0.77	0.54	0.36	0.34

Figure 3.Recommended protein intakes for the Iberian pig during different growth phases.

Conclusion

The overall analysis of the results presented in this review underline the loss of efficiency that undergo the PD process as the pig advances in BW and age. This is reflected in the considerable decrease in the marginal efficiency of PD from the early stages of growth, expressed by a curvilinear function. The decrease in this parameter is more noticeable in the Iberian pig that, therefore, does not follow the growth models developed for conventional or lean pig types. These, and other findings, fully support the use of dietary regimes of lower protein concentration

adjusted to the particular needs of the growth period under consideration, as protein needs suffer a dynamic change along the productive life of the animal. There are several advantages associated to this practice; one of them is the environmental benefit derived from the lesser nitrogenous wastes produced, as all the protein (nitrogen) provided in oversupply will be finally catabolized and excreted; on the other hand, protein reduction in feeds could imply also an economic benefit for the producer as usually protein is one of the most expensive feed ingredients. There are also animal health and welfare considerations as protein excess can cause digestive problems in weaning piglets, and also can be deleterious for Ca metabolism according to our last findings in this area (Nieto et al., unpublished).

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Proteini u ishrani autohtone iberijske svinje

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Rezime

Tačnu procenu uslova potreba životinje je od izuzetnog značaja za uravnoteženu ishranu domaćih životinja. U ovom pregledu se opisuju rezultati niza eksperimenata naše istraživačke grupe, obavljenih tokom poslednjih godina, u cilju procene korišćenje proteina u ishrani iberijske svinje tokom različitih faza njenog proizvodnog ciklusa. Studije koje se bave ishranom i tretmanima/dozama koje uključuju nekoliko izoenergetskih tretmana koji se razlikuju u koncentraciji proteina - sa sličnim aminokiselinskim profilom koji prati koncept idealnog proteina - zajedno sa komparativnim studijama rasta i metabolizma sa konvencionalnim rasama su izvršene. Naša zapažanja pokazuju da je kapacitet ove rase za deponovanje proteina prilično ograničen u poređenju sa konvencionalnim ili mesnatim rasama u sličnim fazama rasta. Oni takođe ukazuju na potrebu za smanjenje koncentracije proteina u ishrani iberijske svinja u skladu sa metaboličkim profilom ove autohtone rase svinja. Mi rezimiramo sve informacije

prikupljene tokom poslednjih petnaest godina i dajemo preporuke o nivou i sastavu proteina u ishrani za čiste rase životinja tokom različitih faza rasta. Adekvatno upravljanje ishranom, naročito u odredbama koje se odnose na protein, poboljšava efikasnost korišćenja proteina u ishrani i dovodi do relevantnih ekonomskih, socijalnih prednosti i za životnu sredinu i za dobrobit životinja.

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IMMUNOCASTRATION IN PIGS

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Invited paper

Abstract: There is a strong initiative in the European Union to stop physical castration of piglets as practiced today and alternatives have been proposed. Among them immune castration; it consists of two vaccinations against gonadotropin releasing hormone (GnRH) and uses the natural immune system of pig for the achievement of castration-like effect. It seems an acceptable alternative to physical castration because it offers a solution for boar taint problem avoiding pain and also aggressive behaviour that is seen when rearing entire males (EM). Moreover, physiological effects of immunocastration are not expressed until seven days after revaccination (V2), usually performed 4-6 weeks preceding slaughter, which allows benefiting from feed efficiency and lean tissue growth of EM through most of the growing-finishing period. However, after V2 a substantial increase in average daily feed intake is observed in immunocastrated pigs (IC) resulting in a progressive decrease in feed efficiency and an increase in body fatness of IC in comparison to EM. Although the vaccine producer recommends an interval of 4 weeks between V2 and slaughter, recent studies indicated that a shorter or longer delay could be considered. Regarding the effectiveness of immunisation, it should be noted that some animals do not react to it, thus there is a need of a fast, simple but effective method for boar taint detection on the slaughter line. The paper gives an overview of immunocastration effects on boar taint, body composition, meat quality, with an emphasis on feeding strategies, advantages and disadvantages of this alternative.

Key words: pig, immunocastration, performance, boar taint

Introduction

Castration of male piglets is a routine practice in pig husbandry with main aim to avoid boar taint; it involves surgical procedure in which testes with epididymides are physically removed from scrotal sack; and in EU countries it is allowed to be performed without anaesthesia or analgesia during the first 7 days of

life *(Council Directive, 2008)*. Castrated pigs are also calmer and fatter and express less sexual behaviour in group housing *(EFSA, 2004)*. Although generally used, this procedure of castration has been criticized as being painful for the piglets *(McGlone et al., 1993; Prunier et al., 2006)*. It may be considered even unnecessary in modern pig production, especially if pigs are raised for meat consumption, where raising entire males (**EM**) would be more efficient *(Bonneau, 1998;Trefan et al., 2013)*. Thus, for welfare and economic reasons a strong initiative (http://boars2018.com) has been developed in the European Union tovoluntary stop surgical castration as practiced nowadays until 2018 and thus a need has emerged for new, alternative solutions for boar taint prevention. Intensive research of alternatives to surgical castration, diagnostic methods for boar taint detection, harmonization of analytical methods of substances responsible for boar taint, and attitudes of stakeholders is being supported by the European Commission (http://ec.europa.eu/food/animal/welfare/farm/initiativesen.htm; According to current indications the most promising alternatives are immunocastration, surgical castration with pain relief, rearing of EM, genetic selection on the reduced boar taint and semen sorting technique with subsequent rearing of female offspring. The latter two methods are sustainable and animal welfare friendly but long-term alternatives, whereas the first three alternatives are suitable for immediate introduction into practice. It seems that for fresh meat consumption rearing of EMis the most interesting alternative, whereas immunocastration,a method that will be discussed in the present review, remains a good alternative in the case of fattening to a higher age and weight.

Immunocastration

Immunocastration uses natural immune system of the pig to produce specific antibodies that bind and neutralise the gonadotropin releasing hormone (**GnRH**) and by that, blocks the hypothalamic-pituitary-gonadal. It effectively inhibits testis growth and sexual steroids synthesis *(Hilbe et al., 2006; Fang et al., 2010; Einarsson et al., 2011)* and can be successfully used as an alternative to physical castration of pigs *(Dunshea et al., 2001)*. According to the manufacturer's instructions, the vaccine should be administered in two doses at least four weeks apart. Although no withdrawal period is needed, it is recommended that the second vaccination (**V2**) is performed four to six weeks before slaughter, in order to ensure clearance of boar taint substances from adipose tissue. However, recent studies demonstrated that a shorter *(Lealiifano et al., 2011; Kubale et al., 2013)* or longer delay could be considered *(Claus et al., 2008; Einarssonet al., 2009)*. At commercial slaughter weights, levels of androstenone and skatole are below sensory threshold already two weeks after V2 *(Lealiifano et al., 2011; Kubale et*

al., 2013) whereas the effect of immunocastration persists for at least 10 weeks after V2 *(Claus et al., 2008; Zamaratskaia et al., 2008; Einarsson et al., 2009)*. This timing is important from the perspective of pig production. The shorter the delay, the more benefit from the boar-like performance can be expected, whereas fluctuations in the demand or other specific market needs could lead to a prolonged delay and pig producers need to be sure that there is no risk of boar taint.

The effects of immunocastration on boar taint compounds and reproductive organs

The results of recent meta-analysis *(Batorek et al., 2012a)* show that the magnitude of the response in efficient immunocastration is by far the largest for reproductive organs and boar taint substances. This has been shown in all studies, despite the high heterogeneity between studies due to different vaccination protocols used. In agreement with *Brunius et al. (2011)*, the effects on reproductive organs are larger for early vaccination than for late vaccination. The largest reduction is observed for seminal vesicle weight *(Bonneau, 2010; Batorek et al., 2012a; Čandek-Potokar et al., 2014)*. This is related to their anatomical structure of liquid-containing vesicles, which can be quickly resorbed, whereas the other accessory glands and testes take more time to regress due to firmer structure. This result supports the suggestion of *Bonneau (2010)* to use seminal vesicles as a diagnostic tool to assess the success of immunocastration at slaughter. Consistent with the regression of the reproductive tract, a loss of functional activity is observed at a histological level *(Falvo et al., 1986; Grizzle et al., 1987, Awoniyi et al., 1988; Kubale et al., 2013)*. Immunocastration reduces the mean diameter of the seminiferous tubules, the number of spermatogonia and spermatocytes, induces the atrophy of the Leydig cells and reduces the weight of glandular tissues and secretory products of accessory sexual glands *(Kubale et al., 2013)*. However, the intensity of changes occurring at a histological level is dependent on the time of immunization *(Einarsson et al., 2011; Kubale et al., 2013)*. The impairment of testis functionality results in the prevention of the accumulation of boar taint compounds in adipose tissue *(Batorek et al., 2012a)* ofimmunocastrated pigs (**IC**). However, as shown in the above mentioned meta-analysis, slight differences between IC and surgically castrated pigs (**SC**) for both substances remain and could perhaps be due to the effectiveness of immunisation. Namely, it should be noted that some animals do not react to the vaccine (so-called "non-responders") due to poor immunological response or technically improper vaccination *(Jaros et al., 2005; Škrlep et al., 2012)*. The number of non-responders is relatively low (1-3%) and is expected to be similar to the incidence of cryptorchidism. However, this

implies a need for simple but effective method for their detection at the slaughter line.

The effects of immunocastration and different feeding strategies on growth performance

Physiologically IC are similar to EM before V2, with no difference in growth performance *(Millet et al., 2011; Batorek et al., 2012a)*. However as observed in meta-analysis *(Batorek et al., 2012a)*, feed intake tends to be higher in IC than in EM, which might be related to an early response to the vaccination in some pigs, shown to have reduced luteinizing hormone (**LH**) and testosterone levels already after the first vaccination (**V1**; *Turkstra et al., 2002*). The majority of IC produces a sufficient quantity of antibodies to neutralize all secreted GnRH shortly after V2. Consequently LH and steroid secretions are suppressed and the metabolism adapts itself in approximately seven days after V2 *(Claus et al., 2007)*. The largest effect observed is a drastic increase in feed intake comparatively to EM (for approximately 450 g/day; *Millet et al., 2011; Batorek et al., 2012a; Dunshea et al., 2013*). This effect was related to the sharp reduction in the production of androgens and oestrogens, known to decrease feed intake *(Claus and Weiler, 1987)*, and to reduced socio-sexual behaviour in IC compared to EM *(Cronin et al., 2003)*. Likewise, feed intake of IC after V2 is slightly higher compared toSC (for approximately 105 g/day; *Batorek et al., 2012a; Millet et al., 2011; Dunshea et al., 2013*). A possible explanation resides in hormonal changes following V2 as well. The absence of testicular steroids and the presence of relatively low amounts of leptin in IC after V2 compared with SC *(Batorek et al., 2012b)* might cause high feed intake in IC, because both oestrogens and leptin are known to reduce appetite *(Claus and Weiler, 1994; Barb et al., 1998)*. Together with the presence of higher insulin-like growth factor I in *ad libitum* feed IC compared to SC *(Claus et al., 2007; Batorek et al., 2012b)*, which contributes to improved feed efficiency, the increased feed intake stimulatesgrowth. Indeed, it was shown that the average daily gain (**ADG**) of IC in the period following V2 is approximately 145 and 117 g/day higher compared to SC and EM, respectively *(Millet et al., 2011; Batorek et al., 2012a; Dunshea et al., 2013)*. Eventually feed efficiency in IC following V2 is improved compared to SC (for approximately 0.10 points; *Millet et al., 2011; Dunshea et al., 2013*) but reduced compared to EM (for approximately 0.35 points; *Millet et al., 2011; Dunshea et al., 2013*). It has also been shown that maintenance metabolisable energy requirements of IC are intermediate between SC and EM *(Labussiere et al., 2013)*. Considering the whole fattening period (V1-slaughter) IC are much more efficient than SC because physiologically they are EM before V2 and also some time after, however they are less efficient and slightly fatter than

EM because they are slaughtered after the transient period is finished, but they exhibit moderately faster growth compared with both SC and EM *(Batorek et al., 2012a)*. These facts initiated the idea to study the effects of restricted feed allowance of IC after V2 with the aim to improve feed efficiency and to avoid increased fatness of the carcass of IC. In that regard, our results *(Batorek et al., 2012b)* show that despite a slower growth of feed-restricted IC, feed efficiency is improved and performance traits are similar to those in *ad libitum* fed EM. On the contrary, *Quiniou et al. (2012)* suggest that feed restriction of IC pigs after V2 has no practical interest, because restricting feed allowance to group-housed IC results in slower growth with no improvement of feed efficiency. The discrepancy in the results of the mentioned studies can perhaps be dueto different experimental conditions in particular the housing system (individual vs. group). However, both studies suggested that restrictive feeding of IC could increase aggressiveness as higher incidence (comparable to EM)of lesions during fattening *(Quiniou et al., 2012)* or prior to slaughter *(Batorek et al., 2012b)* was observed. This may perhaps be due to chronic stress and higher excitability caused by feed restriction *(D'Eathet al., 2009)* and, for that reason, might be questioned from a welfare point of view. Reduced dietary energy content in feed by inclusion of fibre-rich ingredients without limitation of feed allowance might be applied as an alternative.

The effects of immunocastration and different feeding strategies on carcass composition and meat quality

The results of meta-analyses agree that IC have lower dressing percentage than SC (for approximately 2.0%; *Batorek et al., 2012a; Dunshea et al., 2013*) and, although not always significantly, also than EM (for approximately 0.3%; *Batorek et al., 2012a; Dunshea et al., 2013*). Lower dressing percentage in IC has been attributed to the presence of reproductive tract *(Boler et al., 2012; Boler et al., 2014)*, higher gut fill due to increased average feed intake *(Dunshea et al., 2001; Gispert et al., 2010; Boler et al., 2014)*, increased weight of abdominal fat *(Škrlep et al., 2010a; Škrlep et al., 2010b)* and also to the heavier internal organs, namely liver and kidneys *(Pauly et al., 2008; Pauly et al., 2009; Boler et al., 2014)*. Because physiologically immunocastration becomes effective in the last weeks before slaughter, when the transient period after V2 is finished, IC take advantage of boar like growth performance for most of growing-fattening period. Hence they produce leaner carcasses compared to SC (lean content is increased for approximately 1.0%; *Pauly et al., 2009; Millet et al., 2011; Batorek et al., 2012a*) due to increased muscling and decreased fatness. According to *Dunshea et al. (2013)* backfat thickness is approximately 2.64 mm lower in IC compared to SC, but approximately 1.53 mm higher than in EM at slaughter. Latter can be attributed

to high feed intake following V2 that enhances fat deposition and consequently lowers lean content of the carcass in IC compared to EM (for approximately 1.5%; *Pauly et al., 2009; Millet et al., 2011; Batorek et al., 2012a*). However, fat deposition in IC largely depends on the timing of immunization - the length of period between immunization and slaughter *(Lealiifano et al., 2011)* and possibly feeding strategy applied. However, feed restriction in IC had either no significant effect on fat deposition and carcass leanness (*dos Santos et al., 2012; Quiniou et al., 2012*) or only reduced leaf fat weight *(Batorek et al., 2012b)*. The lack of effect of restricted feed allowance on carcass leanness in the above mentioned studies might be related to a shorter duration of the applied restriction. In general, the weight of the main carcass cuts of IC and EM seems similar, with exception of heavier belly weight *(Batorek et al., 2012a)*, which is consistent with the higher fat content of the carcass. Whereas the heavier ham and shoulder weights and the lighterbelly weight in IC compared with SC reported in meta-analysis *(Batorek et al., 2012a)* can be ascribed to the fact that muscles of the fore and hind limbs develop earlier in life than muscles of posture (e.g. loin, belly muscles) and may be less affected by the reduced anabolic potential after immunocastration as discussed by *Pauly et al. (2009)*. The synthesis of published data on meat quality shows that IC do not differ from SC *(Batorek et al.,2012a; Pauly et al., 2012)*, whereas IC present some advantages over EM, namely higher intramuscular fat content and lower shear force *(Batorek et al., 2012a; Pauly et al., 2012)*. Later might be attributed to increased growth rate in the weeks prior to slaughter, resulting in enhanced protein turn-over *in vivo* and increased proteolysis *post-mortem (Therkildsen et al., 2004; Lametsch et al., 2006)*. Although compensatory growth has been proposed to have a positive effect on pork tenderness *(Kristensen et al., 2002)*, neither sensory tenderness nor juiciness were affected by castration method or sex *(Pauly et al., 2012; Trefan et al., 2013)*. Our meta-analysis also indicated that IC might have some disadvantages in meat quality compared with EM *(Batorek et al., 2012a)*, including a higher drip loss, which is consistent with a tendency for lower ultimate pH and higher CIE L (lightness) value. However this was not confirmed by *Pauly et al. (2009)* and *Trefan et al. (2013)*, with exception of lighter meat colour *(Trefan et al., 2013)*.

Conclusions

Physiologically the immunocastration becomes effective in a week following V2, therefore in case of late revaccination during the month preceding slaughter, growth characteristics of IC are similar to those of EM and better production performance (i.e. growth rate, feed intake, feed efficiency and carcass leanness) of IC compared with SC is observed. In regard to meat quality, IC are

more similar to SC than EM. Although the vaccine producer recommends that a four-week interval between V2 and slaughter, recent studies indicated that a shorter or longer delay could be considered. Regarding the effectiveness of immunization, it should be noted that some animals do not react to it, and thus, there is a need of a simple but effective method for their detection on the slaughter line.

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Rezime

Postoji jaka inicijativa u Evropskoj uniji da se zaustavi fizička kastracija prasadi kako se praktikuje danas i predložene su alternative. Među njima je imunokastracija: sastoji se od dve vakcinacije protiv gonadotropin oslobađajućeg hormona (GnRH) i koristi prirodni imuni sistem svinje za postizanje efekta kastracije. Čini se prihvatljiva alternativa fizičkoj kastraciji jer nudi rešenje za problem nepoželjnog polnog mirisa nerasta i izbega bol i agresivnog ponašanja koje se vidi kada se gaje nekastrirani mužjaci (EM). Pored toga, fiziološki efekti imunokastracije nisu izraženi do sedam dana nakon revakcinacije (V2), obično se izvodi 4-6 nedelje pre klanja, što omogućava efikasnost u korišćenju hrane i rast mišićnog tkiva kod EM tokom najvećeg dela perioda porasta-završni period. Međutim, nakon V2 značajan porast prosečnog dnevnog unosa hrane je primećen kod imunokastriranih svinja (IC) što rezultira progresivnim smanjenjem efikasnosti hrane i povećanjem masnog tkiva u telu u odnosu na EM. Iako proizvođač vakcina preporučuje interval od 4 nedelje između V2 i klanja, novije studije pokazuju da bi kraće ili duže odlaganje/produženje ovog perioda moglo da se uzme u obzir. Što se tiče efikasnosti imunizacije, treba napomenuti da neki životinje ne reaguju na nju, tako da postoji potreba za brzim, jednostavnim ali efikasnim metodom za detekciju polnog mirisa nerasta na liniji klanja. Rad daje pregled uticaja imunokastracije svinja na polni miris nerasta, telesni sastav, kvalitet mesa, sa naglaskom na strategije hranjenja, prednosti i nedostatke ove alternative.

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STREPTOCOCCUS SUIS, MOST COMMON SEROTYPES ISOLATED FROM DISEASED PIGLETS IN SOME FARMS IN SERBIA

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Invited paper

Abstract: The aim of this study was to establish serotype affiliation of *S. suis* strains in piglets that died after septicemia and meningitis clinical symptoms similar to those caused by this pathogen. The material analyzed in this study included 104 brain, kidney, joint, lung and blood samples, both part of the organs and swabs from the organs from deceased piglets that had clinical feature of meningitis. Bacterial strains were selected on the basis of colony morphology, catalase negativity, hemolytic characteristics (α haemolysis) they produce on blood agar, and their microscopic appearance. For identification of bacteria, classical and commercial tests API 20 Strep and Rapid ID32 STREP (bioMérieux, France) were used. Serological typing with antisera (Statens Serum Institute, Denmark) specific for capsular *S. suis* antigens (Quellung reaction) was used in order to determine the serotypes of the isolated strains. *S. suis* serotype 2 was the most common among the 78 isolated strains (47,5%), and the only one isolated from all collected samples, serotype 9 was the second most frequent serotype isolated from diseased piglets with the prevalence of 28,2%, and it was not isolated from piglets blood, while other serotypes (1, 3, 4 and 8) were isolated in far less percentage. The results of this study showed that *S. suis* serotypes isolated from clinicaly ill piglets had prevalence similar to the prevalence reported in major European pig producing countries. Prevalence of serotype 2 *Streptococcus suis* was the highest, although it is noted that prevalence of serotype 9 in disesed pigs was remarkably increased since some earlier research in Serbia.

Key words: *Streptococcus suis*, serotype prevalence, diseased piglets

Introduction

Streptococcus suis is a normal inhabitant of the respiratory system of pigs (*Gottschalk et al., 2007*). Contrary to the fact that it is normal inhabitant, *S. suis* is the major pathogen causing significant losses on the pig farms. *S. suis* can often be isolated from tonsils, nasal cavities as well as from genital and gastrointestinal system of healthy animals. Since it is a very good colonizer of the mucosal surfaces, clinically healthy pigs are the main reservoir of infection, and the most important link in the epidemiology of human infections caused by *S. suis*. With almost 100% of pig farms worldwide having carrier animals, *S. suis* is one of the most important bacterial pig pathogen. Transmission of S. suis among pigs is mainly by respiratory route, but also some other routes have important role. From the moment it is first described as the important pathogen, *Streptococcus suis* has been identified in all countries with developed pig industries (*Stanojković et. al., 2011*). *S. suis* can cause diseases in pigs of all age categories, including suckling piglets, older piglets and pigs. *Streptococcus suis* is major pathogen causing meningitis in pigs, but also can cause septicemia, endocarditis, pneumonia and arthritis. Sometimes in per acute cases of infection pigs are found dead with no previously noticed signs of disease. In addition, it is an emerging zoonotic agent responsible for septicemia with or without septic shock, meningitis and other less common infections in humans.

Since first described in Denmark in 1968, over 1600 human cases of *S. suis* infection have been reported with many more probably never diagnosed or misdiagnosed. During the last decade the number of reported human cases due to *S. suis* has dramatically increased, and while most sporadic human cases of infection appear to be due to close occupational contact with pigs/ pork products, particularly in Western countries (farmers, veterinarians, butchers, food processing workers, etc.), two epidemics were recorded in China in 1998 and 2005. As of 2006, the number of human cases reported in Asia has increased. In fact, in some Asian countries, the general population is at risk. *S. suis* is the most common cause of adult meningitis in Vietnam, the second most common in Thailand and the third most frequent cause of community-acquired bacterial meningitis in Hong Kong.

S. suis is encapsulated, gram positive species , that occur single, in pairs or occasionaly in short chains. It has components of cell wall antigens similar to those displayed by group D streptococci. However, *S. suis* is not genetically associated with group D streptococci. *S. suis* is a very heterogeneous species. So far, 35 *S. suis* serotypes have been described on the basis of the composition of the capsular polysaccharide. The organism grows well on media usually used for isolation of streptococci, most frequently sheep blood agar, and forms glistening, round, slightly grey alpha haemolitic colonies. It can growth well in aerobic conditions but the growth is enchanced by microaerophilic atmosphere. Isolation and

identification of strains is relatively easy, especially in the cases of diseased animals. *S. suis* has vary variable biochemical properties (*Stanojković et al., 2014*) and thus must be confirmed by serotyping. Although S. suis can be easily identified by veterinary laboratories that are aware of pathogen, many human veterinary laboratories misidentify it as enterococci, Streptococcus pneumoniae, viridans streptococci or even Listeria monocytogenes and mainly by use of rapid multitest biochemical kits (*Gottschalk et al., 2010*). This confusion may have led to the misdiagnosis of S. suis infections in the past. Serotyping is the only accurate method for definitive diagnosis of S. suis infection. This can be done by two methods. First, by serological method that is performed by either co-agglutination, capillary precipitation test or Neufeld's capsular reaction using reference antisera. Second method is serotyping using PCR method in which CPS (capsular) genes are amplified by either simplex or multiplex PCR. Polymerase chain reaction (PCR) tests have been used to directly detect S. suis DNA from samples with a high sensitivity. Some S. suis isolates do not agglutinate with any of the antisera directed against 35 serotypes and these are identified as non-typable isolates.

During the last 12 years, more than 4500 serologically confirmed strains recovered from diseased pigs have been reported. Globally, the most dominant serotypes isolates from clinical cases in pigs are serotypes 2,9,3,1/2 and 7, while 15,5% were so called non-typable strains. However, there is clear geographical distribution of serotypes.

In Canada the most prevalent serotype is serotype 2 while in United State serotype 3 is the most prevalent. In these countries there is only slight difference in percentages of prevalent strains, demonstrating similar distribution of serotypes when data from Canada and the USA are combined. Both, serotypes 2 and 3 are the most prevalent from diseased pigs with 24,3% and 21,0% prevalence respectively, followed by serotypes 1/2, 8 and 7 (*Goyette-Desjardins et al., 2014*). This can be explained by easy and freely movement of animals from United states to Canada and vice versa.

In South America , all results came from Brasil, stating that serotype 2 being the most prevalent with 57,6% reported cases followed by serotypes 1/2, 14, 7 and 9. In Asia majority of results regarding serotype affiliation came from China and South Korea. In China the most prevalent serotypes of infected pigs are in decreasing order of prevalnce, serotype 2, 3, 4, 7 and 8. On the contrary in South Korea serotype 2 had a prevalence of only 8,3% , the same ase serotypes 8 and 33 while the most dominant were serotypes 3 and 4 with 29,2|% and 20,8% respectively, while serotypes 16 and 22 had distribution of 4,1%. Other Asian countries reported many human cases of disease but strains isolated from pigs only refer to slaughterhouses and healthy pigs. Similary, in Japan there have been reported 10 human S. suis cases reported but studies on the distribution of isolates

from ill pigs have not been reported lately and all of the research dates before 1987 year. In Cambodia, Philippines, Laos and Singapore, human cases were diagnosed recently but there are no data avilable on the epidemiology of S. suis infections in pigs.

In Europe, most of the S. suis serotype distribution reports date before year 2000. S. suis serotype 2 was the most common in clinical cases in Italy, France and Spain, whreas serotype 9 was more frequent in the Netherlands, Germany and Belgium. Recent conducted reaserch on serotype distribution in Spain suggest that serotype 2 is no longer the most prevalent serotype, and that serotype 9 is the one most frequently isolated from diseased pigs. Behind serotype 9 is serotype 2, followed by serotypes 7, 8 and 3 (*Luque et al., 2010*). In Netherlands, serotype 9 was the most prevalent in data collected between 2002-2007 followed by serotypes 2, 7, 1 and 4 (*Schultsz et al., 2012*). Contrary to the fact that serotype 9 becomes most prevalent in some countries, there were no human cases reported that were associated with this serotype. In Belgium and United kingdom serotype 1 was the predominant in ill pigs while in Denmark serotype 7 was the most frequent one. In Southern Europe serotype distribution was only done in Serbia where serotype 2 was the only serotype found in piglets that had clinical symptoms of meningitis (*Stanojkovic et al., 2012*). Beside that various *S. suis* serotypes were found in healthy animals (*Stanojković, 2012*).

Multilocus sequence typing (MLST) distinguishes a large number of genotypes while using genetic variations that accumulate very slowly, in house keeping genes, and has allowed global and longterm epidemiology for many important meningitis-causing bacteria by determining the ST's present within a population. In 2002 , King et al. established a model of MLST for S. suis using seven house-keeping genes (*cpn*60 , *dpr*, *rec*A , *aro*A , *thr*A, *gki* and *mut*S).Many laboratories through the world use MLST model to determine sequence types (ST) of isolated S. suis strains. MLST allows gathering genetic diversity information of S. suis strains within the different serotypes. More recently, studies have begun combining data obtained from MLST with the presence or absence of different S. suis virulence-associated markers at the gene and protein levels including the suilysin (SLY, encoded by the *sly* gene), muramidase-released protein (MRP, encoded by the *mrp* gene), extracellular factor (EF, encoded by the *epf* gene) and different pili in order to compare ST's data with phenotypic characteristics.

The aim of this study was to establish serotype affiliation of *S. suis* strains in piglets that died after septicemia and meningitis clinical symptoms similar to those caused by this pathogen.

Materials and methods

The material analyzed in this study included 104 brain, kidney, joint, lung and blood samples, both part of the organs and swabs from the organs from deceased piglets that had clinical feature of meningitis. Samples were transported in trypton soy broth (Oxoid, England) within 2 h of sampling and incubated for 4 hours at 37 °C, and by using standard microbiological methods, were inoculated on Columbia CNA agar with 5% sheep blood (bioMérieux, France), and incubated for 24 h in conditions at 37 °C. Bacterial strains were selected on the basis of colony morphology, catalase negativity, hemolytic characteristics (α haemolysis) produced on blood agar, and their microscopic appearance. For identification of bacteria, classical and commercial tests API 20 Strep and Rapid ID32 STREP (bioMérieux, France) were used. Serological typing with antisera (Statens Serum Institute, Denmark) specific for capsular *S. suis* antigens (Quellung reaction) was used in order to determine the serotypes of the isolated strains.

Results and Discussion

From the 104 tested samples, 78 strains of *S. suis* were isolated. Table 1 shows the number and percentage of determined serotypes of *S. suis.*

Table 1. The number and percentage of isolated *S. suis* serotypes.

Serotypes described	Number of isolated serotypes	Isolated serotypes (%)
S. suis serotype 1	1	1,3
S. suis serotype 2	37	47,5
S. suis serotype 3	3	3,8
S. suis serotype 4	4	5,1
S. suis serotype 7	9	11,5
S. suis serotype 8	2	2,6
S. suis serotype 9	22	28,2

S. suis serotype 2 was the most common among the 78 isolated strains (47,5%), and the only one isolated from all collected samples samples. This result is similar to results that were obtained in most European countries before year 2000, China, Canada, Brasil and results all taken globally. On the contrary serotype 2 had prevalence in South Korea of only 8,3% and it was second frequent isolated serotype in the United states, Netherlands, Germany and Belgium. Also, serotype 2 is no longer dominant in Spain as it was in our research. Serotype 9 was the second most frequent serotype isolated from diseased piglets with the prevalence of 28,2%, and it was not isolated from

piglets blood. We ackonowledge that this serotype had higher distribution than expected because in other research in Serbia prevalence was not in this high percentage. This is in correlation to serotype distribution in Spain where serotype 9 prevalence became most isolated *S. suis* serotype. Also, these results follow pattern in most European countries in increased isolation frequency of *S. suis* serotype 9 from diseased pigs. Other serotypes were isolated in far less number. Serotypes 1, 3, 4 and 8 were isolated in one, three, four and two piglets respectively. Serotype 1 was isolated only from joint sample, serotype 3 from joint and kidney samples, serotype 4 from all samples except lung, and serotype 8 was isolated from brain and kidney samples. Results referring to serotypes 1, 3, 4 and 8 are similar to those in Europe except these serotypes in some countries have different order in distribution frequency. For example, in Spain the second most common serotype is serotype 2 followed by serotypes 7, 8 and 3, while in the Netherlands second common serotype 2 is followed by serotypes 7, 1 and 4. In China serotypes following most common serotype 2 are serotypes 3, 4, 7 and 8. Significant difference is noted in South Korea where serotypes 3 and 4 are the most common ones, in in United States where serotype 3 is the most frequently isolated, in Belgium and United kingdom where serotype 1 had highest distribution and in Denmark where serotype 7 was the most prevalent serotype.

Regarding results in this research we acknowledge that these results are similar to those of other authors from Europe. Although, differences can be found in prevalence of serotypes 2 and 9 which change their places as the first and the second most common serotypes in most important pig producing countries in Europe.

Conclusion

The results of this study showed that *S. suis* serotypes isolated from clinicaly ill piglets had prevalence similar to the prevalence reported in major European pig producing countries. Prevalence of serotype 2 *Streptococcus suis* was the highest, although it is noted that prevalence of serotype 9 in diseased pigs is increasing. One thing that has not been done yet in Serbia is *S. suis* multilocus sequence typing (MLST) in order to distinguish different genotypes isolated from pigs as well as from human infections and in some future reaserch it is essential to obtaine these kind of results.

Streptococcus suis, najčešće izolovani serotipovi kod obolele prasadi na nekim farmama u Srbiji

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Rezime

Cilj ovog istraživanja je bio da se utvrdi serotipska pripadnost sojeva S. suis kod prasadi koja su uginula posle kliničkih znakova septikemije i meningitisa sličnih onima koje izaziva ovaj patogen. Materijal korišćen u analizama se sastojao od uzoraka krvi, mozga, bubrega i pluća, kako delova tih organa ,tako i briseva istih. Bakterijski sojevi izolovanih bakterija su selekcionisani na osnovu morfologije kolonija, negativnosti u testu katalaze, hemolize na krvnom agaru (alfa hemoliza) i mikroskopskog pregleda. Za identifikaciju bakterija klasični i komercijalni testovi API 20 Strep i Rapid ID32 STREP (bioMérieux, France) su korišćeni a seološka tipizacija sojeva S. suis je serumima specifičnim za kapsularne antigene ove bakterije (Statens Serum Institute, Danska) takozvanom Quellung reakcijom. *S. suis* serotip 2 je u našem istraživanju među 78 izolovanih sojeva bio najfrekventniji izolovani serotip (47,5%), i jedini koji je bio izolovanih iz svih uzoraka, serotip 9 je bio sledeći drugi po prevalenciji (28,2%) i nije bio izolovan iz krvi obolelih svinja dok je prevalencija ostalih izolovanih serotipova (1, 3, 4 and 8) bila znatno niža. Rezultati ove studije su pokazali da je S. suis izolovan kod klinički obolelih prasadi imao prevalenciju sličnu onoj ustanovljenoj u Evropskim zemljam sa razvijenim svinjarstvom. Iako je prevalencija serotipa 2 *S. suis* bila najviša utvrđeno je da se distribucija serotipa 9 ove bakterije značajno povećava.

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STORAGE OF PERISHABLE WET BY-PRODUCTS FED BY RUMINANTS

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Invited paper

Abstract: The authors reviewed the current situation with respect to a wide range of wet by-products that are available and applicable in ruminant nutrition. The main aim of the review was to show the short- and long term storage background, details, opportunities, difficulties and potential future of the wet by-products worldwide.

Key words: wet by-products, aerobic stability, sugar beet pulp, wet brewers' grain, wet corn gluten feed

Introduction

This paper reviews the current situation with respect to a wide range of wet by-products that are available for ruminant nutrition with some details of the short- and long term storage opportunities and difficulties. The global annual yield of the major by-products can be estimated to be in the range of 1000 million tonnes/annum. Whilst many of these by-products are of low dry matter content (range 15-25% DM) they offer a massive feed resource particularly with the current global demands on land and water for the production of food, feed and fuel. There are some other factors that have made this topic of increasing importance over the past decade.

1. The dramatic increased use of corn and cereals for the production of bio-ethanol as a liquid fuel has increased the supply of various types of wet grain by-products.
2. The increase in biogas plants that need a continuous supply of energy rich feeds for the production of biogas.
3. There is an opportunity to save arable lands for food-, bio-ethanol and biogas industry using the wet by-product in animal nutrition.
4. Most of these wet by-products (as the 'traditional forages'), are available only at a certain period of the year making long-term storage a real need.

5. Wet by-products have a very short shelf life if appropriate storage conditions are not used, due to the deterioration instigated by the growth of undesirable microorganisms.
6. Fermentation, as a conservation method, has better economic prospects compared to energy demanding drying.
7. Nutritive value of some wet by-products are higher than conventional forages, so as part of a balanced diet, they can make a significant contribution to ruminant diets and therefore production efficiency.

Short term storage of wet by-products

Short-term storage (2-14 days) of wet by-products (brewers' grains, wet corn gluten feed) is associated with the periodic delivery of fresh material from breweries to farms. Storage facilities are usually concrete or plywood boxes, but unprotected piles are not uncommon as a method of keeping wet brewers'grains or wet corn gluten feed during the feeding period between delivery dates. In some cases, storage during this interval has resulted in mold and yeast growth as well as dry matter breakdown in surface layers of the grains. Losses appear to vary with the length of storage, the type of storage facility and the ambient temperature during the storage period (*Allen et al., 1975a,b*). There is an inverse relationship between dry matter losses associated with the storage of high-moisture material and the degree of air-tightness achieved (*Allen et al., 1975a*).

2.1. Aerobic spoilage on the surface and subsurface processes of wet by-products during short term storage

Wet grain by-products are generally stored in horizontal structures. Covering of the silos with plastic film of at least 150 µm or oxygen barrier film sealing immediately following filling is essential for maximal recovery of nutrients during extended storage periods (>10 days), but is also recommended even for short term storage.

2.1.1. Deterioration processes in aerobic top layer of untreated wet by-products during short term storage

Deterioration is more pronounced in the upper, aerobic layer (soft material on the top: 0-10 cm depth) compared to the lower, subsurface layer in wet brewery grain. It was confirmed by an elevated ammonia-N content in the upper layer at 20°C (ammonia-N in total N: aerobic layer 2.31 % *vs.* subsurface layer 0.85 %) and the poor microbial status of the untreated wet brewery by-product (molds: aerobic

layer 4.88 log10 CFU/g *vs.* subsurface layer 2.72 log10 CFU/g) (*Orosz et al., 2010*).

Extensive mold growth, discoloration and dry matter deterioration were noted in fresh wet brewers' grains at 23% dry matter and 4.7% DM total nitrogen in uncovered piles (placed outside on polyethylene sheeting) during a 14-day storage period for treatments of 85% formic acid at 0.20% and 0.40%; propionic acid and formic-propionic mixture (1:1) at 0.20, 0.30, and 0.40%; molasses at 2% and the untreated control, respectively (*Allen et al., 1975a*).

The wet corn gluten feed is another fairly perishable material (dry matter: 400-420 g/kg, crude protein: 200-220 g/kg DM), but in many cases short term storage is the only option due to periodic delivery. In a trial (*Orosz, 2007 unpublished*), the aerobic surface of wet corn gluten feed (at 10 cm depth) and the subsurface layer (50 cm depth) were stable during 6 days according to the measured pH and temperature (open container with a capacity of 1 m^3 placed in a barn protected against sunshine and rain, filled with non-packed fresh corn gluten feed). On the 14th sampling day, mold growth, discoloration were found on the top, while the wet CGF in the subsurface layer (50 cm depth) visually was similar to the initial stage .The temperature and pH results on the 14th day showed accelerated spoilage in the top 10 cm (initial temperature: 20 °C *vs.* day 14: 47 °C) and pH (initial pH: 4.2 *vs.* day 14: 5.5), while the subsurface layer had not changed significantly compared to the start (initial temperature: 20 °C *vs.* day 14: 22.1 °C) and pH (initial pH: 4,2 *vs.* day 14: 4,1). It can be concluded that the inner wet CGF was significanty more stable due to limited access of oxygen compared to the top, aerobic layer (Day 14 pH 10 cm: 5.5 *vs.* pH 50 cm: 4.1, $p \leq 0.05$; temperature 10 cm: 47 °C *vs.* temperature 50 cm: 22.1 °C, $p \leq 0.05$)

2.1.2. Undesirable processes in the subsurface layer of wet by-products during short term storage

The subsurface layers in wet brewers' grains were more stable in a trial (*Orosz et al., 2010*) shown by lower ammonia-N content and better microbial status (aerobic bacteria count: aerobic layer 6.48 $\log_{10}$ CFU/g *vs.* semi-anaerobic layer 5.03 $\log_{10}$ CFU/g, $p \leq 0.05$, molds: aerobic layer 4.88 $\log_{10}$ CFU/g *vs.* semi-anaerobic layer 2.72 $\log_{10}$ CFU/g, $p \leq 0.05$,). Otherwise, a considerable acetic acid fermentation and ethanol production were found in the subsurface layer compared to the upper (aerobic), untreated material (acetic acid: aerobic layer 1,37 g/kg DM *vs*. anaerobic layer 6,66 g/kg DM $p \leq 0.05$, ethanol: aerobic layer 0 g/kg DM *vs.* anaerobic layer 1.84/kg DM, $p \leq 0.05$, fermentation products: aerobic layer 3.03 g/kg DM *vs.*

anaerobic layer 10.56 g/kg DM, p≤0.05) (*Orosz et al., 2010*). Similar results were found in another trial, where a rapid increase in acetic acid concentration in the subsurface samples (75 cm depth) was detected for brewers' grains in the untreated control (*Allen et al., 1975*). The process can be described as non-controlled fermentation by yeasts and undesirable facultative anaerobic bacteria. As aerobic deterioration in the semi-anaerobic subsurface and inner layers (on 20°C for 10 days) were not so intensive as in the aerobic top layer (significantly less aerobic bacteria and mold count in the subsurface layers), thus causing a lower animal health risk during short term storage.

In a trial (*Orosz, 2007 unpublished*) the wet CGF in the subsurface layer (50 cm depth) was quite stable, but significant microbial activity was found showing deterioration processes had already started (initial stage aerobic bacteria count: 2.9 $\log_{10}$ CFU/g, mould count: 2.0 $\log_{10}$ CFU/g, day 14th aerobic bacteria count: 4.6 $\log_{10}$ CFU/g, mold count: 5.2 $\log_{10}$ CFU/g, yeast count 5.8 $\log_{10}$ CFU/g, p≤0.05, respectively). A lactic acid concentration of 7 g/kg DM was observed in the subsurface layer (50 cm depth) in wet CGF on the 14th day of storage.

2.2. Inhibitory effect of different treatments on deterioration of wet by-products during short term storage

2.2.1.Effect of different treatments on aerobic spoilage in the top layer of wet by-products during short term storage

Some chemicals are effective at preventing growth of undesirable microorganisms, heating and spoilage of wet by-products. Propionic acid and its salts, formic acid and its salts, ammonia, urea, sulfur dioxide, sodium benzoate and potassium sorbate can be options as additives applied just to the top (aerobic) layer, or to the entire bulk of wet by-products during short term storage (maximum 10 days) before feeding (*Buchanan-Smith et al., 2003*). Toxigenic fungi (molds) generally grow under humid, warm, aerobic conditions. Therefore wet by-products are particularly at risk

(i) in the field (the grain can be infected by molds and contaminated by mycotoxins before harvest),
(ii) during the industrial process (mycotoxin concentration will be increased in the by-product due to starch and protein extraction),
(iii) on farm, during the short term storage in open-air (often non-covered) horizontal piles before feeding (especially in humid and warm tropical-subtropical climates and continental regions in the summer time when the temperature is above 20°C).

Propionic acid is an active fungicide that prevents the mold proliferation and mycotoxin contamination of wet by-products. Propionic acid levels required to preserve high moisture corn are 4.5-17.5 g/kg wet material for the moisture content in the range of 180-400 g/kg. The propionate solution at pH 4.86 was as effective as pure propionic acid (pH = 1.70), it is likely that the propionate ion does contain considerable antimicrobial activity. Levels of propionate, rather than propionic acid, and propionate-acetate mixtures that are required for effective preservation of wet by-products are likely to be slightly greater than the pure acid alone. Lower application rates may be used for shorter (anaerobic) storage times for grain by-products compared to acid application for long term anaerobic storage. Schneider et al. (1995) found that 5 g/kg dose propionic acid applied to wet brewers' grain (DM: 185-300 g/kg) preserved the material for periods of 90 days. Solubilization of nitrogen and formation of NH_3 can be decreased by propionic acid application relative to untreated wet by-products during long and short term storage (*Schneider et al., 1995; Sebastian et al., 1996*). The propionic acid reduces the lactic acid production in the subsurface layers, but does not eliminate it (*Schneider et al., 1995; Sebastian et al., 1996*). Formic acid is also an important component of mixtures to preserve wet brewers' grain, since acidification, mold and yeast inhibition are important aspects of preserving these by-products (*Allen et al., 1975*). Mixture of 2.5 g/kg propionic acid and 2.5 g/kg formic acid added to wet corn gluten feed (438 g/kg DM) preserved it at 26.5 °C for periods up to 21 days.

Orosz et al. (2010) found that a dose of 0.3% propionic acid-formic acid (1:1) treatment in the upper soft and aerated layer of wet brewers' grain (top 0-10 cm depth) did not inhibit the deterioration compared to the control (pH 6.36 *vs*. control pH 5.97, respectively, $p \leq 0.05$). Moreover, the added propionic acid (0.15%) concentration decreased by 96% in the aerobic layer over 10 days compared to the initial concentration and was lower than the propionic acid concentration in the anaerobic layer (aerobic layer: 5.9 g/kg DM, anaerobic layer: 10.1 g/kg DM, $p \leq 0.05$). Presumably, undesirable aerobic bacteria and molds were able to use the propionic acid as substrate in the dose of 0.15% in aerobic circumstances. Therefore application of 0.3% dose of propionic and formic acid mixture (1:1) is not recommended as an application for the upper surface treatment, because it presumably supplies substrate for aerobic undesirable micro-organisms causing accelerated deterioration compared to the untreated wet by-product in the aerobic surface.

Two rates (0.20 and O.40%) of formic acid and the high rate (0.40%) of propionic acid were unable to reduce the amount of surface spoilage (*Allen et al., 1975*).

Acid treatments were effective in inhibiting the deterioration process in the aerobic layer (10 cm depth) of wet CGF stored in open tanks for 14 days (*Orosz, 2007 un-*

published). The temperature in the top layer of wet CGF was significantly lower when applying propionic acid treatment (dose: 0.25%) and ammonium-propionate (control: 47.4 C, propionic acid 0.25%: 34.5ºC, ammonium-propionate 0.1%: 41.3, ammonium-propionate 0.2%: 36.5; $p \leq 0.05$). Propionic acid treatment (P dose: 0.25%) and ammonium-propionate (AP dose: 0.2%) were effective at reducing the aerobic spoilage of the top in wet CGF (stored in open tanks without packing) causing significant differences in pH compared to the untreated control (control pH: 5.5, PA treatment pH: 4.3; AP treatment pH 4.2; $p \leq 0.05$). The lower dose ammonium-propionate treatment (AP dose: 0.1%) was not effective in spoilage inhibiton.

The acids and acid mixture applications are irritants and corrosive, therefore ammonia-buffered products can be used, as less harmful additives. The efficiency of these ammonia-buffered acid mixtures were investigated (*Orosz et al., 2010*) in anarobic and semi-anaerobic conditions in wet brewers' grain during short term storage (10 days). The buffered acid mixture (dose: 0.5%, composition: 50.4 % formic acid, 29.7% ammmonium-formate, 18.1% propionic acid, 1% mono-propylene-glycol and 0,8 % water) inhibited the spoilage compared to the untreated wet brewery grain in the upper (top 10cm) aerobic layer (aerobic bacteria count of control: 6.48 $\log_{10}$ CFU/g; 0.3% PA:FA 1:1 treatment: 6.48 $\log_{10}$ CFU/g; 0.5% buffered mixture treatment: 4.12 $\log_{10}$ CFU/g; $p \leq 0{,}05$). Otherwise, it was less effective than the treatment of 0.5% un-buffered acid (PA:FA 1:1) mixture (aerobic bacteria count at dose 0.5% PA:FA 1:1 treatment: 3.00 $\log_{10}$ CFU/g, and 0,5% buffered mixture treatment: 4.12 $\log_{10}$ CFU/g, $p \leq 0.05$). The aerobic mesophil bacteria- and mold count was significantly higher in the top 10 cm layer treated with the buffered acid mixture compared to the dose of 0.5% of acid mixture (PA: FA 1:1) in wet brewers' grains.

Since inner layers are more stable microbiologically than the top, and whole treatment of the wet by-products on the farm has a potential health hazard for the farm-worker and is expensive, it is recommended to apply a surface acid or acid mixture treatment by spraying keeping the rules of health and safety. Treatment of the wet by-product pile surface with 0.5% acid mixture (PA:FA 1:1) can be effective to inhibit undesirable aerobic microorganism growth during short storage (for a maximum 10 days) (*Orosz et al., 2010; Orosz, 2007 unpublished*). Lower doses can be effective, but it is recommended to consider that significant amounts of acids will volatilize from the surface. For this reason, covering the piles is recommended even for short term storage of wet by-products.

2.2.2.Effect of different treatments on spoilage in the subsurface layer of wet by-products during short term storage

A rapid increase in acetic acid in the subsurface samples (75 cm depth) was detected for brewers' grains in untreated control and treated with the low and medium rates (0.20 and 0.30%) of both propionic and formic-propionic acid, 2.0% molasses. The two rates (0.20 and O.40%) of formic acid and the high rate (0.40%) of propionic acid were effective in reducing subsurface deterioration of wet brewers' grains (*Allen et al., 1975a*).

The 0.3% dose of acid mixtures (PA:FA 1:1) had a beneficial effect on deterioration in the semi-anaerobic layer compared to the control. The dose of 0.5% was significantly more effective in mold inhibition than the dose of 0,3% acid mixtures (PA:FA 1:1) in the subsurface layer (0.3%: AEMB 4.53 $\log_{10}$ CFU/g *vs.* 0.5%: AEMB 3.0 $\log_{10}$ CFU/g, $p \leq 0.05$). The effect of buffered acid mixture (0.5%, PA:FA 1:2.5) on wet brewers' grains profile was similar to the dose of 0.5% of acid mixture (PA: FA 1:1) in the subsurface layer (*Orosz et al., 2010*).

It was found that 0.5% of acid mixture (PA: FA 1:1) could inhibit effectively (at 20°C and 20% DM content of wet brewers' grains) the deterioration process compared to the control in both aerobic and subsurface layers (*Orosz et al., 2010*). There were none significant differences between the aerobic and subsurface layer characteristics in the case of 0.5% acid (PA: FA 1:1) treatment.

Long term storage of wet by-products

Before discussing the long storage it is worth briefly reminding ourselves of a few management factors affecting the storage of wet by-products, be they ‘forages’ or ‘concentrates’. *Woolford (1986)* stated that the presence of air is the ‘Achilles heel’ of the ensiling process. This is probably even more true of wet by-products than any other ensiled feed. Carbon-dioxide is heavier than air and it has a tendency to gravitate to the bottom of the silo from where it escapes, thus leaving a void, filled by air generally at the top of the silo (*Honig, 1991; Williams et al., 1997*). The oxygen drawn into the silo is rapidly used producing more CO_2, therefore a continuous gaseous exchange process can be detected between O_2 and CO_2. The extent depends on a number of factors such as silo type (*Jiang et al., 1989*), compaction density, dry matter and particle size (*Pitt et al., 1991; Pitt, 1986; Williams et al., 1997*). So, if we now consider the case of ensiling wet by-products there are a number of important additional risk factors worthy of note.

1. The by-product has lost most, if not all, of the biological activity with respect to respiration, such that oxygen entering the store will be entirely available for the growth of aerobic microorganisms increasing the population size of these undesirable organisms.
2. The quantity ensiled/stored is generally limited, giving it a larger surface area:volume ratio making it more vulnerable to CO_2 egress and O_2 ingress. Therefore the plastic tube method would be the more efficient silo type for many wet by-products than other silo types with higher capacity and slower filling rates.
3. As the wet by-products have a low dry matter content (15-50 % DM range), and the structure is variable their consolidation on farm is a challenge. For this reason again the plastic tube system is a potential solution as the prefered silo type in order to obtain anaerobic conditions as quickly as possible, with little human effort required. There is little or no data on effluent production on ensiling of the different by-products. Grass, at 15% dry matter can produce an effluent volume of 200 l/ton diminishing to 0%, when the dry matter content reaches 30% (*Bastiman, 1976*). Most of the wet by-products fit this dry matter range. Obviously it is not correct to extrapolate data derived from whole plant silages (such as grass) to processed by-products. There are many different factors (particle size and the hygroscopic properties), that will exert their effects on effluent production. Another unknown aspect is the composition of the effluent and its effect on nutritive value of the wet by-product silage.
4. The procedures for sealing the bunker silo is a challenge, allowing a greater chance of carbon dioxide egress and oxygen ingress and overall gaseous exchanges with these type of wet by-products.
5. The hygenic status of the wet by-products recieved on farm is variable. Even in the vicinity of the factory (1-200 km), signs of aerobic deterioration can be found in many cases. The temperature measurement can be a monitoring tool on the farm. The fermentation efficiency will be affected in an aerobically deteriorated wet by-product.
6. Soil contamination is also a high risk in the case of leafy by-products derived from the arable (eg. sugar cane top) and when the factory or the farm store the by-product temporarly in open-air.
7. There may be remnants of different chemicals used during the industrial processes to extract starch, sugar etc, which may have an effect on the fermentation process and aerobic stability.
8. Some of the by-products are treated with acids or salts (eg. 3 l/ton propionic acid treatment of wet CGF (*Orosz, 2015 unpublished*) by the factory in order to prevent aerobic deterioration during preliminary storage

and transport. This is likely to have a positive effect on the long term storage processes and aerobic stability.

The following section serves as a 'taster' of the information available on some of the more common by-products.

3.1. Long term storage of wet by-products derived from the sugar industry

3.1.1. Sugar beet pulp and molassed sugar beet pulp

3.1.1.1. Fermentation characteristics of wet sugar beet pulp

The ensilage of sugar beet pulp (SBP) and molassed sugar beet pulp (MSBP) has been carried out for over 70 years. Olsen described the fermentation process as early as 1951, the author compared pulp ensiled alone or with the addition of 4% molasses. The addition of molasses (*Olsen, 1951*) resulted in an almost pure lactic fermentation with *Streptobacterium plantarum* and *S. casei* dominating the initial process. However, after 2-3 months only *Betabacterium breve* could be found. This fermentation resulted in losses of 8 %, as a result of CO_2 production. In the case of wet sugar beet pulp without molasses, the butyric acid fermentation dominated as a result of Clostridia activity and dry matter losses of 12-15% were measured due both to CO_2 and effluent production.

Kilic and Saricicek (2011) investigated the use of 8 different ensiling treatments (1.5 l glass laboratory silos, 60 day) and the effect on nutrient content, pH, physical factors and *in vitro* digestibility of SBP. The treatments were the following: control; AIV system; urea at 1% FW of SBP; formic acid at 2.5 kg/ton FW, Maize-all inoculant applied at 1 x 10^5 CF/g FM (*L.plantarum, L. salivarius, P. acidilactici* + Amylase), Sil-All inoculant applied at 1 x 10^5 CF/g FM (*L. plantarum, L. salivarius, E. faecium, P. acidilactici* + cellulase, hemicellulase, pentonase and amylase); 0.5 kg/ton FM dry sodium formate; 5-7 kg/ton mix of sodium formate and formic acid. The ash content was significantly higher in the AIV treatment at 167 g/kg DM (all other treatments 54.8 to 77.5 g/kg DM), while the urea treatment was intermediate at 107 g/kg DM. The pH was the lowest in the AIV treatment at 1.45, all other treatments ranged from pH 3.17 to 3.55. The urea treatment significantly increased crude protein levels to 212 g/kg DM from *circa* 115 – 120 g/kg (inoculated and untreated SBP), while the formic acid and AIV treatments were significantly lower at 88 and 99 g/kg, respectively. The energy content was the greatest in the inoculated silages and lowest in the AIV treatment. The authors concluded (*Kilic and Saricicek, 2011*) that the AIV treatment was not advisable, but the other treatments all had merits. Including the urea treatment, which did not affect fermentation adversely and had the benefit of increasing crude protein content. In a study examining a wide range of additives *Li and Lu (2009)* concluded that SBP silage made without additives had a poorer fermentation quality than those ensiled with 7 different silage inoculants. Sodium sulphite and

sodium sulphate treatment also showed an improvement compared to the contol SBP.

Ensiled SBP was shown to increase bio-ethanol production by 50% compared to fresh SB (*Zheng et al., 2012*). In this study they showed no benefit of inoculating the SBP before ensiling with *L. fermentum* compared to the untreated control, as this inoculum is hetero-fermentative this is probably not a surprising result. This study also indicated that the ensiling process not only offers a storage solution, but can significantly increase production efficiency of some processes.

3.1.1.2. Technical aspects of wet sugar beet pulp ensiling

The wet sugar beet pulp can be stored in a heap if covered to exclude air, and stored for up to two weeks during October-January. For longer storage it should be ensiled. Ensiling of pressed sugar beet pulp has shown that the conservation in plastic tube silos can be excellent even for long storage periods of time (up to 18 months) and this technique offers a cost effective storage method (*Wagner et al., 2009*). Due to the rapid filling the fermentation can begin quickly and due to the rapid feed-out rates the risk of aerobic spoilage is low. Since its introduction in 1993/94 more than 1 million tonnes of pressed sugar beet pulp across Europe have been stored in plastic tube silos according to Budissa Bag Technologies (*Engelhard et al., 1994*).

3.2. Long term storage of wet brewers' grains

3.2.1. Fermentation characteristics of wet brewers' grains

Wet brewers grains (WBG) are very perishable and the fermentation can be a problem due to the low dry matter- and high crude protein content. Ensiling results in a low organic acid concentration (LA: 1.69 %DM, AA: 2,69%DM) in untreated WBG after 90 days of ensiilng according to *Schneider et al. (1995).* Despite the WBG having a rather low pH (pH 4,1). Schneider et al. (1995) conducted a number of studies examining different preservation methodologies with wet brewers'grian (WBG). The studies examined the use of inoculants (1 x 10^5 CFU/g compared to 1 x 10^6 CFU/g), acids (propionic acid 0.5% w/w) and absorbent materials with sugar beet pulp pellets (inclusion rate: 15% FM), on the preservation of WBG across 5 different trials. The DM of the WBG ranged between the experiments from 18.5% to 30 % indicating high variability of the WBG. *Schneider et al. (1995)* showed that the use of an inoculant containing a mix of Homofermentative Lactic acid bacteria improved fermentation quality over untreated silage as measured by increased levels of lactic acid and reduced levels of both acetic and butyric acids. The level of inoculation of either 1 x 10^5 CFU/g compared to 1 x 10^6 CFU/g also had a significant effect, with the higher inoculation rate being significantly better. The higher inoculation rate (1 x 10^6 CFU/g) significantly reduced butyrate concentrations after 57 days of ensilage compared to the untreated control and 1 x

10^5 CFU/g inoculation. In their further studies (*Schneider et al., 1995*), propionic acid applied at 0.5% w/w (on a FM basis) did not indicate an inhibition of the fermentation. Propionic acid application (0.5% w/w) had a positive effect on the ratio of lactic to acetic of 1:8 compared to 1:2.6 in the untreated WBG. They also found significant differences in total acid content (PA 0.5%: 50.5 g/kg DM vs. untreated: 39.5 g/kg DM). The use of beet pulp (BP) pellets (*Schneider et al., 1995*) at 15% inclusion rate (FM) increased the dry matter content from 29.5% (untreated control) to 40% in the BP treated WBG. The inclusion of the BP reduced the crude protein content from 31.9% to 22.9%. The BP had a debatable effect on fermentation quality. When BP (15% FM) treatment was combined with an inoculant (homofermentative bacteria, 10^5 CFU/g), the lactic acid fermentation was improved (LA:AA ratio BP 15% and inoculant:1.5, BP 15% alone: 0.95).

Orosz et al. (2008, un-published) mixed WBG with wilted chopped lucerne (331 g/kg DM, 174 g/kg DM crude protein, 431 g/kg DM NDF, chop size: 50% - 8-19 mm) in a ratio of 40 WBG:60 L and 60 WBG:40 L (based on fresh weight) and baled with a special baler (Göweil LT Master, high density bales: 1,1 ton/bale, density: 846 kg FM/m^3). The lucerne applied as a structural fiber for bale formation allowed a long storage period of 6 months to be achieved. Fermentation quality was very poor (Table 1): pH 6.0, low lactic acid-, very high acetic- and rather high butyric acid content were found with extremely low LA:AA ratio after 90 d of ensilage. Ammonia-N content was elevated indicating intensive protein breakdown. The mold counts were low probably due to the combination of the high levels of undesirable acids and the rapid baling ensiling technology that quickly reduced the oxygen concentration.

Table 1. Fermentation parameters of wet brewers' grain ensiled with fresh chopped lucerne in high density bales (*Orosz et al., 2008a, un-published*)

(n=3)	pH		Lactic acid		Acetic acid		Propionic acid		Butyric acid		Total acid	
			g/kg DM		g/kg DM		g/kg DM		g/kg DM		g/kg DM	
	mean	std	mean	std	mean	std	mean	std	mean	std	mean	std
40%WBG: 60% L	6.0a	0.2	11.4a	4.1	47.7a	2.9	10.7a	0.6	22.2a	1.8	95.7a	3.6
60%WBG: 40% L	6.0a	0.1	16.8b	0.9	42.9b	1.5	10.7a	0.7	26.9b	0.3	106.7b	1.7

	Ethanol		LA:AA		NH_3-N/ total N		Aerobic bacteria		Mould	
	g/kg DM		g/g		%		lg_{10} CFU/g		lg_{10} CFU/g	
	mean	std	mean	std	mean	std	mean	std	mean	std
40%WBG: 60% L	5.6a	0.3	0.2a	0.1	22.8a	0.77	5.85a	0.77	0.61a	0.12
60%WBG: 40% L	4.5b	0.2	0.4a	0.0	31.6b	0.48	5.03b	0.51	0.20b	0.35

Different letters indicate significant difference $p \leq 0.05$

3.2.2. Technical aspects of wet brewers' grain ensiling

The brewers' grain can easily be consolidated to a high density due to its particle size and low dry matter content. Well preserved brewers' grains in bunker silos can be found on farms. The silo should have proper drainage to collect runoff. Brewers grains silage fermentation is complete by 3 weeks and the ensiled material will keep for 6 months, and even more if a silage additive is used. However, the plastic tube system is more common worldwide as a proposition to store WBG. Advantages of this technology are the flexible capacity, the quick filling and long term maintainance of the anaerobic conditions. Although, gas and effluent production can be hazards during the first 1-3 days of fermentation. The propionic acid treatment (1-3 l/ton) with or without ground cereal or maize silage addition can also be found on farms. *Orosz et al. (2008, un-published)* recommended the special (high density) baling (as flexible volume silo type) for the long term storage of WBG mixed with structural fiber sources. Packing and ensiling characteristics can be improved by blending the wet brewers grains prior to ensiling with dry materials such as dry forage, bran or hulls, or with a source of fermentable carbohydrates such as molasses or cereal grains (*Blezinger, 2003*).

3.3. Long term storage of wet by-products derived from the starch or ethanol production

3.3.1. Wet corn gluten feed

The wet corn gluten feed (WCGF) is a rather perishable by-product due to its 60% moisture content, therefore short-term storage (7-10 days) as fresh wet CGF may cause an animal health risk on the farm. Fermentation of wet CGF is a solution for long term storage, but there is limited information about the fermentation quality and aerobic stability of the ensiled wet CGF after the silo is opened.

3.3.1.1. Fermentation characteristics of wet corn gluten feed

Orosz and Kapas (2010) examined the ensiling characteristics of WCGF. The WCGF had a low organic acid content on the 30th day of fermentation (Exp 1: 12.1 g/kg DM, Exp 2: 8.5 g/kg DM), accompanied by pH of 4.7- 4.8). However, LA:AA ratio was relatively good (Exp 1 LA:AA: 9.7, Exp 2 LA:AA: 4.0). Ethanol production was considerable in the WCGF (Exp 1 ET: 19.0 g/kg DM, Exp 2 ET: 31.3 g/kg DM). Summarizing, the WCGF had a low fermentation intensity with a very high ethanol concentration (*Orosz and Kapas, 2010*). In this trial *Orosz and Kapas (2010)* examined the ensiling characteristics of WCGF in combination with 10% dry ground corn grain or 20% maize silage. Maize silage improved fermentation intensity of WCGF (organic acid 12.1 g/kg DM *vs* 18.2 g/kg DM), however significantly increased the acetic acid production and reduced the LA:AA ratio compared to the WCGF control (9,7 *vs* 2.2). The dry corn increased the lactic acid concentration (6.6 g/kg DM *vs.* 9.3 g/kg DM) and significantly reduced the ethanol conent of the WCGF (31.3 g/kg DM *vs* 23.4 g/kg DM) between the 14-30 day. However, acetic acid production was more intensive compared to the control (1.7 g/kg DM *vs.* 2.9 g/kg DM).

Orosz et al. (2008b, unpublished) investigated the use of silage additives in the preservation of WCGF. This study involved the ensilage of WCGF for 30 days either alone or after treatment with one of the following additives: a chemical mixture (59% formic acid, 20% propionic acid, 4.3% ammonium formate and 2.5% potassium sorbate) applied at 3 rates either 4, 5 or 6 l/t (FPA4, FPA 5, FPA 6) or a silage inoculant (INOC: containing *L.plantarum, P. acidilactici, L. salivarius, E. faecium* plus enzymes: cellulase, hemicellulase, amylase applied at 2 x 105 CFU/g) or the above inoculant with K sorbate and Na benzoate (INOC + PRES: K-sorbate, Na-benzoate, applied dose: 250g/ton AF, 3 litre water/ton). The authors concluded that chemical treatments had a negative effect on lactic acid production, the acetic acid concentration did not change considerably, while there was a significant decrease in the ethanol concentration (Table 2). The residual sugar content was significantly higher in the WCGF treated with acid mixtures showing a less intensive fermentation processes. The authors did not find significant differences between the dose rates applied. They concluded that silage additive containing mixtures of formic- and propionic acids (59% formic acid, 20% propionic acid, 4,3% ammonium-formate, 2,5% K-sorbate), at a rate of 4 litre/ton was sufficient to inhibit yeast growth and ethanol production.

Table 2. Fermentation parameters of wet corn gluten feed treated with different acid mixtures and inoculants *(Orosz et al., 2008b, unpublished)*

n=3		pH	Lactic acid	Acetic acid	Total acid	T/E	Ethanol	NH3-N	Total sugar
			g/kg DM	g/kg DM	g/kg DM		g/kg DM	% of total N	g/kg DM
WCGF	mean	4.81a	9.1a	1.3a	10.4a	7.3a	25.6a	4.2a	15.1a
	std.	0.01	0.4	0.1	0.4	0.8	2.1	0.3	1.7
FPA4	mean	4.17b	7.2b	1.3a	8.5b	5.7b	0.9c	4.4a	52.4b
	std.	0.03	1.0	0.0	1.0	0.9	0.1	0.1	2.0
FPA5	mean	4.24b	8.2b	1.3a	9.5b	6.1b	2.0d	4.6a	49.3b
	std.	0.02	0.6	0.0	0.6	0.4	0.6	1.1	2.0
FPA6	mean	4.16b	6.8b	1.3a	8.0b	5.4b	0.7c	4.9b	49.6b
	std.	0.01	1.5	0.1	1.5	1.0	0.1	0.2	3.2
INOC.	mean	4.83a	7.4b	1.2a	8.6b	5.9b	19.3b	4.8b	12.3a
	std.	0.02	1.1	0.1	1.2	0.7	1.9	0.1	2.6
INOC + PRES	mean	4.83a	7.8b	1.5a	9.3a	5.5b	15.7b	4.5a	15.2a
	std.	0.02	1.1	0.4	1.1	1.4	8.9	0.1	4.0

Different letters indicate significant difference $p \leq 0.05$

FPA4, FPA 5, FPA 6: 59% formic acid, 20% propionic acid, 4.3% ammonium formate and 2.5% potassium sorbate applied at 3 rates either 4, 5 or 6 l/t, INOC: containing *L.plantarum, P. acidilactici, L. salivarius, E. faecium* plus enzymes: cellulase, hemicellulase, amylase applied at 2 x 105 CFU/g) or the above inoculant with K sorbate and Na benzoate, INOC + PRES: (*Enterococcus faecium, Pediococcus acidilactici, Lactobacillus salivarius, Lactobacillus plantarum*, K-sorbate, Na-benzoate, applied dose: 250g/ton AF, 3 litre water/ton).

Orosz et al. (2008a, un-published) mixed WCGF with wilted chopped lucerne (331 g/kg DM, 174 g/kg DM crude protein, 431 g/kg DM NDF, chop size: 50% - 8-19 mm) at a ratio of 70% WCGF:30% L and 60% WCGF:40% L (based on fresh weight) and baled with a special baler (Göweil LT Master, high density bales: 1,05 ton/bale, density: 846 kg FM/m^3). Fermentation quality of the mixture 70% WCGF:30% L was intensive (Table 3): pH was 4.3 associated with 84 g/kg DM total acid and 62.7g/kg DM lactic acid after 90 d of ensilage. The fermentation quality was not so good in the case of the 60% WCGF:40% L ratio (pH 4.4, total acid: 95.7 g/kg DM, acetic acid: 32.3 g/kg DM, ammoniaN in total N: 13.2%). Butyric acid was not found in any case. Microbial composition was advantagous and showed anaerobic stability in the high density bales.

Table 3. Fermentation parameters of wet corn gluten feed ensiled with fresh chopped lucerne in high density bales *(Orosz et al., 2008a, un-published)*

n=3	**pH**		**Lactic acid**		**Acetic acid**		**Propionic acid**		**Butyric acid**		**Total acid**	
			g/kg DM		g/kg DM		g/kg DM		g/kg DM		g/kg DM	
	mean	std	mean	std	mean	std	mean	std	mean	std	mean	std
70%WCGF: 30% L	4.3a	0.0	62.7a	10	20.8a	1.1	2.2a	0.1	0a	0	84,1a	11,1
60%WCGF :40% L	4.4a	0.0	60.9a	11.3	32.3b	3.2	2.6b	0.1	0a	0	95,7a	8,7
	Ethanol		**LA:AA**		**NH3-N/ total N**		**Aerobic bacteria**		**Mould**			
	g/kg DM		g/g		%		lg_{10} CFU/g		lg_{10} CFU/g			
	mean	std	mean	std	mean	std	mean	std	mean		std	
70%WCGF: 30% L	6.5a	0.4	3.0a	0.4	10.8a	0.45	4.55a	0.07	0.75a		0.26	
60%WCGF :40% L	7.2a	0.6	1.9b	0.5	13.2b	0.34	3.88b	0.48	0.69a		0.12	

Different letters indicate significant difference $p \leq 0.05$

3.3.1.2. Aerobic stability of wet corn gluten feed

Orosz and Kapas (2010) found that the WCGF aerobic stability was rather poor (49 hours/1°C and 39 hours/1°C) during aerobic deterioration studies (after 30 days of fermentation). Maize silage (20%) increased the mold proliferation compared to the control WCGF, while dry corn decreased the proteolysis (ammonia N%: 7.0 *vs* 5.8) during the aerobic deterioration stage. Maize silage had an undesirable harmful effect on the aerobic stability (49 hours/1°C, *vs* 28 hours/1°C), while dry ground corn significantly improved the aerobic stability of WCGF (39 hours/1°C *vs* 84 hours/1°C).

In a latter trial *Orosz et al. (2013)* found, the mixture of formic- and propionic acids (application rate of 4 l/ton, 5 l/ton, 6 l/ton dose, respectively) were effective in maintaining the low pH and low ethanol concentration during the aerobic deterioration phase compared to the control. Homofermentative bacteria inoculation at ensiling of WCGF did not have a positive effect on aerobic parameters (pH, acetic acid, ammonia-N, aerobic bacteria, molds), moreover it increased significantly the ethanol concentration compared to the control during the aerobic deterioration phase. Combination of homofermentative bacteria and preservative salts (Na-bensoate and K-sorbate) did not have any significant effect on aerobic parameters (pH, acetic acid, ethanol, ammonia-N, aerobic bacteria, moulds). The aerobic stability of the ensiled WCGF was investigated by monitoring the time taken for the temperature to rise to 1^{o}C above ambient temperature (*Orosz et al., 2013*) Chemical treatment at 5 and 6 l/t gave the longest aerobic stability of 157 and 135 h respectively. Formic- and propionic acid treatment at an application rate of 6 litre/ton did not have any additional effect on aerobic stability compared to 5 litre/ton. This was followed by the chemical at 4l/ton (90 h) and the combination of inoculant and chemical preservatives (76 h). The untreated (36h) and homofermentative inoculant (36h) were unstable after exposure to air.

Summarizing, the silage additive containing the mix of formic- and propionic acids (59% formic acid, 20% propionic acid, 4,3% NH4-formiate, 2,5% K-sorbate), at an application rate of 5 litre/ton is recommended to apply for fermentation of WCGF in order to improve aerobic stability after silo-opening.

3.3.1.3. Technical aspects of wet corn gluten feed ensiling

The WCGF could be consolidated easily to a high density, but a density of 443 and 477 kg DM/m^3 caused high weight losses (8,8% and 8,4%, respectively) due to intensive gas and effluent production (*Orosz and Kapas, 2010*). Maize silage increased the weight losses compared to the control WCGF (+1,1%). Dry ground corn (10%) reduced the weight losses (-1,8%) compared to the control (8,4% vs 6,6%, $p \leq 0,05$). The plastic tube system is sensitive to gas production, therefore it is recommended to reduce the density to 370-390 kg DM/m^3. *Orosz et al. (2008, un-published)* recommended the special (high density) baling (as a flexible volume silo type) for the long term storage of WCF mixed with structural fiber sources providing almost instantaneous and excellent anaerobic conditions. Wet corn gluten feed stored in a tube silo for one year maintained its composition. In cold climates, freezing temperatures actually extend the storage life of wet corn gluten feed and it was even possible to store unprotected wet corn gluten feed on the ground in winter (North Dakota) with little spoilage for up to three to four weeks.

However, high summer temperatures reduce freshness to only three to four days, causing palatability problems (*Schroeder, 2010*).

Conclusion and future research needs

This paper has attempted to collect disparate information on numerous wet by-products produced in Europe. There is a huge quantity of these wet by-products available. Additionally, that as part of balanced diet, they can make a significant contribution to ruminant diets and therefore production efficiency. However, when comparing the published work on grass, legumes, whole crop cereal, maize and sorghum and the wet by-product literature, the research published on effective storage is somewhat 'ad hoc'. Much of the cited work has been conducted for feeding experiments and has not examined the details of the storage and fermentation processes that can have such a big influence on the feed intake and utilization of the product in the animal. This paper will hopefully serve as a conduit for researchers to examine these processes in deeper detail and the research to be conducted in a structured and methodical way.

There are a number of key challenges for the future of wet by-product preservation:

1. Low dry matter content and short shelf life of many of the wet by-products.
2. The effect of the factory processing on the feed value/storage efficiency.
3. Proximity of the supplier and end-user (duration of shipment and the deterioration processes).
4. Effect of intermediate storage (on the field or at the factory) before transportation to the end-user.
5. Problems such as microbial and chemical contamination (soil, heavy metals, mycotoxins, acids and salts) need to be investigated.
6. Methodologies for both short and long term storage, including additives and silo technology developments may increase the value of such products.

So, in final conclusion, there is much more research to be done worldwide, for a better and deeper understanding of the wet by-product preservation, including safe and efficient utilization in ruminant nutrition.

Skladištenje kvarljivih vlažnih nusproizvoda u hrani preživara

Sz. Orosz, D. R. Davies

Rezime

Autori daju pregled trenutne situacije u vezi sa širokim spektrom vlažnih nusproizvoda koji su dostupni i primenjuju se u ishrani preživara. Glavni cilj pregleda je bio da se pokaže pozadina kratkoročnog i dugoročnog skladištenje, detalje, mogućnosti, poteškoće i potencijalnu budućnost vlažnih nusproizvoda širom sveta.

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ASPECTS OF LUCERNE PROTEIN VALUE IN CONTEMPORARY ANIMAL FEEDING

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Invited paper

Abstract: Various methods to evaluate the optimal phase for lucerne cutting are described in the article, which is the most important condition for the maximal use of its potential nutritive value. With the use of Mean Stage by Weight (MSW) it is possible to predict the nutritive value of lucerne with high precision. The complexity of MSW method brought to the development od the modified system suggested by domestic authors, called Mean Stage by Fresh Weight (MSFW), which is providing simpler and faster use of this system in the field. In several foreign and domestic investigation it was confirmed that the development phase and vegetation cycle have higher influence on total protein content than on protein fraction ratio, which is in fact the main factor that influences protein utilization. Numerous results show that the highest impact on protein fraction ratio and on the degree of their utilization have the methods for lucerne preservation and the way it is used. The biggest problem is occurring in lucerne ensiling process, where it is needed to provide some measures to control the proteolytic processes, which may decrease the protein utilization. Also, the use of different lucerne based feeds imply the adequate ration balancing, and the proper physical form, as suggested by the contemporary nutritive requirements and recommendations.

Key words: lucerne, development phase, vegetation cycle, protein fractions.

Introduction

Lucerne *(Medicago sativa L.)* is the most important perennial legume used in animal feeding, based on its yield and its nutritive value but also on the area where it is produced both in Serbia and in the World. Some of its "imperfections" are that this species is not possible to produce on acidic soils *(Kammes et al., 2008)*, it has some antinutritive compounds *(Đorđević and Dinić, 2007)*, and high value for anion/cation balance (DCAD) which may have influence on occurrence of milk fever *(Stojanović and Grubić, 2008)*. Also, the most often way it is used, as hay, is the least favorable choice compared to other possibilities (green mass, silage

and haylage, dehydrated mass), taking into account the price and the fact that the hay is the most variable feed considering its chemical composition and nutritive value *(Đorđević et al., 2010a; 2011a)*. Aside from high protein content, the use of lucerne (as dehydrated mass) in the non-ruminant feeding is limited due to depressive influence of fiber on diet digestibility, while the use of lucerne protein concentrate is very expensive solution, which is why this feed is replaced with synthetic amino acids *(Đorđević et al., 2012b)*.

Although *Katić et al. (2002)* mention that potential yield of lucerne can be 60-80 t ha^{-1} and about 20 t ha^{-1} of dry matter, according to the official statistical data *(Statistical Office of the Republic of Serbia, 2014)* in 2014 it was produced 4032 kg ha^{-1} of hay in our country, which is way below its potentials. The reason for that is certainly in improper agrotechinc methods. Also, it is evident that the lucerne is cut too late, when its nutritive value is decreased due to the process of lignification and decrease in protein content, which is happening when the highest dry matter yield is the main goal. Proteins and fiber fractions are the most important parameters of nutritive value in lucerne when formulating diets for ruminants, and they are in negative correlation *(Božičković et al., 2014)*. Because of that lucerne was widely investigated in order to determine the optimal moment for its use. However, the moment for use is not influencing nutritive value but also duration of lucerne use, which has the influence on the exploitation price of this plant culture *(Marković, 2015)*, which is further complicating the explained problem. According to *Crasi et al. (2001)*, when lucerne is cut at the beginning of flowering (10% plants in flowers) the best compromise between yield, nutritive value and exploitation duration is obtained. In order to provide high quality and yield in lucerne the *American Society of Agronomy (2004)* is recommending first cutting in lucerne in the phase of butonization, and following cuts when there is 10 to 25% of flowers. However, *Božičković et al. (2012)* show results of their investigation where the decrease of crude protein content during the bud development phase can be 8% CP in absolute values. All this shows that that the decision of optimal phase for lucerne cutting is a very complex problem, which demands the development of precise and in practice simple method for accurate evaluation of lucerne nutritive value. Also, it is very important to take into consideration the significant variability of various protein fraction content in different development phases and cycles, which in many ways influence the utilization of total proteins.

Methods for determining the optimal phase for utilization

Many investigations confirm that with the advance of lucerne maturity (age) its chemical composition is changed and nutritive value is decreased, along

with the increase in total yield. The reason for that is in the increase of the stem content in plant mass (particularly after flowering) and the increase in lignin content of the plant *(Marković, 2015)*. Earlier cutting is providing lucerne with higher nutritive value but with smaller yield and reduced carbohydrate reserves in the roots which are necessary for regeneration in the next vegetation cycle. The plant is using reserve carbohydrates from the root until the new leaves are formed and start photosynthesis. After cutting this process lasts two to three weeks, or until lucerne achieves 10-15 cm in height. From that moment the plant is beginning to recover carbohydrate reserves in the roots and their amount is increased until the plant reach the full bloom stage. With the more frequent cutting lucerne is not attaining the adequate regeneration of carbohydrate reserves which is decreasing yield and duration of lucerne fields *(Kallenbach et al., 2002; Orloff and Putnam, 2006)*. In optimal conditions the minimal interval between first and second or third cutting should be 30-50 days, which depends on climate and lucerne (cultivar, diversity etc.) *(Katić et al., 2007)*. Especially it should be taken into account that the time of autumn (last) cutting has significant influence on the duration of lucerne field *(Katić et al., 2004)*.

The lucerne age (in days) measured from the first day of growth (after the winter dormant period or cutting) cannot be reliable parameter for evaluation of its nutritive value. The variations are occurring between cuts and between years, and the main influence have the weather conditions, which are very variable within and between different years *(Božičković, 2014)*. According to *Fick et al. (1994)*, in spite of those facts some of the first mathematical models for evaluation of lucerne nutritive value were based on its age in days. In those early investigations where lucerne age in days was used, the linear equations were mostly used to evaluate the amount of digestible organic matter, digestible dry matter and crude protein (CP) and also possible daily dry matter (DM) intake. According to *Reid (1973)* the relation between nutritive value and lucerne age in days, in their investigation, required calibration for every location, while equations were under the strong influence of season and weather conditions. This means that those equations could be used only for one location and during the ongoing season. In practical conditions, as a method to estimate the optimal moment for cutting, the evaluation of lucerne maturity is performed according to prominent phases in lucerne development. The prominent phases are usually the beginning of budding, budding, beginning of flowering and flowering. However, for the precise estimation of its nutritive value, the lucerne maturity needs to be numerically expressed.

The moment of lucerne cutting is most adequately determined based on its chemical composition and nutritive value. The most precise method for that is chemical analysis in the laboratory, but it is not practical because if its duration and price. NIRS (*Near-infrared spectroscopy*) technology provides the rapid obtaining

of results about lucerne nutritive value, but the limiting factor for wider use of NIRS is in its relatively high price. Also, for successful use of NIRS technology it is necessary to dry plant materials, which is extending the time for obtaining chemical composition. *Kalu and Fick (1981)* formulated a method to evaluate basic parameters of lucerne nutritive value before cutting based on the precisely determined development stage (MSW – *Mean Stage by Weight*). In their investigations *Kalu and Fick (1981; 1983)* gave equations for estimation of crude protein (CP), *in vitro* true digestibility (IVTD), neutral detergent fiber (NDF), acid detergent fibre (ADF) and lignin (ADL) which had coefficients of determination of 0.99, 0.98, 0.95, 0.90, 0.95 respectively. In spite of its great potential for evaluation of nutritive value in lucerne MSW is considered relatively complicated procedure which was limiting its field use. Namely, according to *Kalu and Fick (1981)* for determination of MSW value it is necessary to know 10 development phases of lucerne and the samples have to be dried before measurements are taken in laboratory. *Božičković et al. (2013)* suggested a modified method for MSW determination, which is possible to perform in the field. This new way of MSW determination is called *Mean Stage by Fresh Weight* (MSFW). The idea of this modification by *Božičković et al. (2013)* is that it is based on the use of freshly cut lucerne development phases, which improved the speed of data obtaining and the procedure itself is simplified. On the other side, the equations for prediction of basic parameters of nutritive value showed that between MSW and MSFW there is practically no differences *Božičković et al. (2013)*. The suggested modification is enabling much wider use of mean morphological stage in lucerne for evaluation of its nutritive value.

Changes in the protein and protein fraction quantity in lucerne depending on the investigated factors

All over the world in publications devoted to animal nutrition lucerne is described as plant species that is often providing most of the protein in ruminant diets, but with the necessary amount of structural carbohydrates and other nutrients *(Peyraund et al., 2009*). In the beginning animal needs were described as crude nutrients (crude protein, crude cellulose, crude fat – *Henneberg and Stohmann, 1859*) while contemporary recommendations demand very comprehensive knowledge of certain fractions of proteins, nonstrucural and structural carbohydrates, digestibility, interactions of certain components, physical effectivity of fiber and other data *(Grubić et al., 2014*). Based on this details it can be concluded that physical and biological availability of nutrients in feeds is as important as is their chemical composition *(Mertens, 2011)*.

The amount and quality of protein in lucerne depend firstly on the development phase, cut, cultivar and variety, but also on the conservation process and the way it is used in animal feeding *(Đorđević et al., 2012a)*. Aside with well known decreasing trend in protein quantity during its development, the differences in the speed changes of protein content between certain vegetation cycles is important for animal feeding practice. Those differences are important for the decision when to cut certain growth phases during vegetation. In connection to that *Božičković et al. (2012)* investigated the changes in CP quantity in three vegetation cycles during 2010. During the experiment, in the first vegetation cycle the weather was very cold and rainy, which slowed down the lucerne growth. The cold and rainy lasted until the middle of the second vegetation cycle (mid June), after which the sudden change occurred and the weather was quite hot, which lasted to the end of the second vegetation cycle and all though the third vegetation cycle. Due to the influence of weather there were a notable differences in the decreasing trend in CP during vegetation. The first vegetation cycle had more sudden decrease in % of CP until the 40-th day of vegetation, when it was settled on about 17% CP, which lasted until the end of vegetation. Second and third vegetation cycles had more balanced decrease in % of CP. On Figure 1 between the scattered vertical lines is the interval from the early bud to early bloom phase. During the first vegetation cycle the significant decrease in % CP was observed in that period. Namely, from 34-th day of vegetation, which was determined as the early bud phase the plant mass had 26.63% CP, while in the early bloom phase it had 17.12% CP (Table 1). During the early bud phase in the first vegetation cycle the decrease in % of CP was about 9.5% (in absolute values) or 36% (in relative values). In the second and third cycle this decrease was much smaller, about 5 and 4% (in absolute values). It should be noted that in the first vegetation cycle the % of CP decreased to the lowest level exactly in the phase which is recommended as optimal for cutting. If inevitable mechanical damage (in leaves) which occur in hay making is added, the CP content in the first lucerne cut would be additionally decreased. This is one of the main reasons why it is recommended to prepare silage or haylage from the first lucerne cut, while other cuts are better for hay making *(Đorđević and Dinić, 2003)*. Also, the use of appropriate machines (conditioners) decrease mechanical losses and contribute to the preservation of nutritive value in lucerne hay, which is the most often produced feed from this culture *(Đorđević et al., 2010b)*.

One of the characteristics in legume proteins, aside from favorable amino acid composition, is their rapid degradation in the rumen, which is influencing the efficiency of nitrogen utilization. Total amount and ratio of degradable and undegradable protein is one of the main elements in modern recommendations for ruminant nutrition. The optimal ratio of degradable and undegradable protein is required in order to provide nitrogen requirements of rumen microorganisms (from the degradable part), but also to provide the host animal with protein, actually in essential amino acids (from undegradable part and microorganisms) (*Grubić et al.,*

2003). A number of parameters influence the ratio between those fractions, among which the development phase is quite important (*Yu et al., 2004)*, while there is also some influence of cultivar *(Tremblay et al., 2002)*. Aside from *in sacco* (*in situ*) method, the undegradable protein content can be determined with chemical analysis. NDICP (*Neutral detergent insoluble crude protein*) is protein fraction of the diet which is practically undegradable in the rumen, while ADICP (*Acid detergent insoluble crude protein*) is the fraction which is completely indigestible in the intestines.

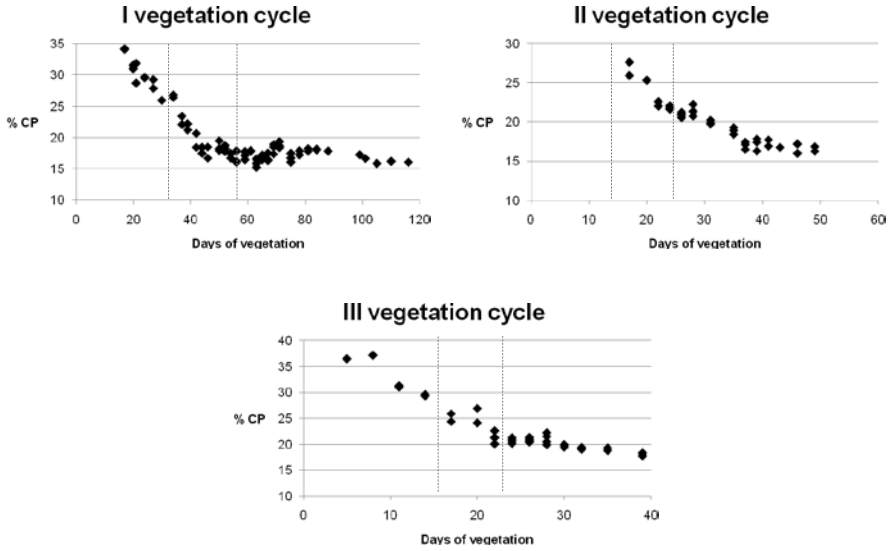

Figure 1. Changes in CP (% DM) during vegetation in I, II and III vegetation cycle. Interval between early bud and early bloom is marked with dotted lines *(Božičković et al., 2012)*

Table 1. Mean value of %CP (DM basis) in the early bud and early bloom stage of development during vegetation cycles *(Božičković et al., 2012)*

Vegetation cycle	Stage of development	Sampling date	Days from beginning of vegetation	Number of samples	%CP mean value
I	Early bud	25.4.2010.	34	2	26.63
	Early bloom	19.5.2010.	59	3	17.12
II	Early bud	10.6.2010.	17	3	26.81
	Early bloom	17.6.2010.	24	3	21.87
III	Early bud	18.7.2010.	17	2	25.17
	Early bloom	23.7.2010.	22	4	21.35

According to CNCPS system which was developed in the Cornell University *(Fox et al., 2004)* feed CP, according to the speed of degradation, can be divided in five fractions (A, B_1, B_2, B_3 and C). Fractions A and B_1 are rapidly degraded in the rumen and consist of non-protein nitrogen and soluble protein. Fraction B_2 is true protein and its degradation rate depends on passage rate. Fraction B_3 is neutral detergent insoluble crude protein (NDICP) from which an acid detergent insoluble crude protein (ADICP) is subtracted. On the other side, according to CNCPS system, ADICP is protein fraction C. According to *Chalupa and Snifen (1994)*, the degradation speed of fractions B_1, B_2 and B_3 is 200-300, 5-15 and 0.1-1.5 %/h, respectively, while their illeal digestibility is 100, 100 and 80 %. The ruminaly undegradable fraction is mostly formed parts of undegraded fractions B_2 and B_3 and complete fraction C, which is not digestible both in the rumen and intestines.

Sniffen et al. (1992) note that fraction B_2 is the single largest protein quantity, and that in lucerne it is about 41%, while fraction B_1 is 51.6% according to *Elizalde et al. (1999)*. The mentioned authors in their investigations determined that the development phase had the highest impact on total amount of CP than on the amount of certain fractions in lucerne. In his dissertation *Marković (2015)* determined that during the lucerne development there was an increase in fractions B_1 and C ($p<0.01$). In the same experiment, from first to fourth lucerne cut there was an increase in the amount of CP, A and B_3 fraction, with the decrease in fraction B_2 ($p<0.01$). In his extensive investigations *Božičković (2014)* did not find significant influence of mean morphological phase, or vegetation cycle on the amount of undegradable protein. Based on that he concluded that the mean morphological phase cannot be used to estimate the amount of undegradable protein in lucerne (Figure 2). Based on the data shown in the Figure 2 it can be concluded that there are no differences between vegetation cycles in the investigated protein fractions. There was a trend of ADICP increase with the advance of vegetation, while there was no clear trend for NDICP. On the other side, *Marković (2015)* shows the results of his experiment where there was significant increase in the NDICP amount in latter development phases in lucerne ($p<0.01$).

Aside from the above mentioned, the processing or conserving has important influence on ruminant feeding. According to *Grubić et al. (1992)* the highest percent of degradable protein has early cut lucerne hay (18%) and lucerne silage (23%). This is why it is important to properly balance the ration which has some of those feeds, with adequate source of undegradable protein *(Grubić et al., 2001; 2005)*.

The process of lucerne conservation has major influence on protein fractions, not only because of ensiling process but also because of specific properties of this plant species and biochemical processes that occur in the silage. In comparison to other legumes, alfalfa proteins are exposed to the greatest extent to hydrolysis *(Đorđević et al., 2004)*. These processes are most intense

immediately after ensiling, although evident for months *(Fairbairn et al., 1988)*. Products of enzymatic degradation of proteins are peptides, free amino acids and ammonia. In the rumen of ruminants these products decompose faster than real proteins, and the ultimate degradation product is ammonia, which may not be fully utilized for microbial protein synthesis. The above products are part of soluble, i.e. degradable proteins, whose content is very well defined in contemporary norms for dairy cow nutrition *(Grubić et al., 2003)*.

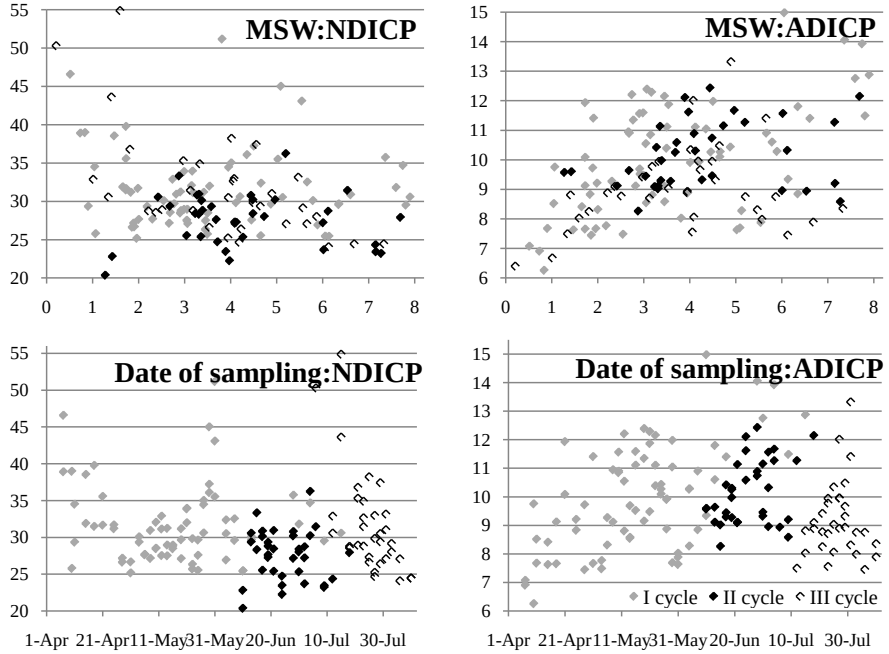

Figure 2. The changes in NDICP and ADICP content (shown as g/kg DM) in green lucerne in connection to the age of the sample (as MSW) and sampling date (*Božičković, 2014)*

All green feed is characterized by increased content of soluble proteins. As stated by *Slottner and Bertilsson (2006)* in living plants 75-90% of the total amount of nitrogen is in the form of true protein, while in silages it is only 30 - 50%. Experimentally significant differences are demonstrated in the degree of solubility between different legumes. According to *Broderick (1995)* other legumes are characterized by a low degree of solubility of proteins compared to alfalfa due to the higher presence of condensed tannins *(Julier et al., 2003)*. *Jones et al. (1995)* report that during ensiling in alfalfa 44-87% proteins are hydrolyzed, while in red clover only 7 - 40% of proteins are hydrolyzed. However, red clover contains no tannins, but still characterized by a low degree of proteolysis during ensiling *(Grabber, 2009)*. *Sullivan and Hatfield (2006)* found that the lower level of

proteolysis in red clover silage compared to alfalfa, can be explained by the existence of buffer soluble proteins - polyphenolic oxidase. This enzyme in the presence of oxygen reacts with o-diphenol building the highly reactive O-quinone, which with other suitable molecules, such as proteins, build polymers *(Parveen et al., 2010)*.

Various methods are used in order to maximally control the process of nitrogen matter degradation during the legume ensiling process, such as wilting, carbohydrate stimulation, inoculation and chemical conserving *(Nadeau et al., 2000; Guo et al., 2008)*. Aside from these technologies the cultivars of legumes are selected to have lower degradability *(Broderick et al., 2004)*, and also genetically manipulations were done with the same purpose *(Getachew et al., 2009)*. The simplest and cheapest procedure is the practice of wilting, which is compulsory measure when ensiling legumes *(Dinić et al., 1999)*. Increase in dry matter in ensiled material reduces the overall microbial activity, as well as enzymes. As a result, the increase of pH values occurs (as a result of lower production of lactic acid), and reduction of the amount of ammonia and soluble nitrogen (Table 2).The increase of dry matter in wilted material also enables greater consumption of dry matter, which is particularly important for high producing animals (*Đorđević et al., 2002)*.

Table 2. Content of nitrogen fraction (gkg^{-1} N) and parameters of biochemical changes in alfalfa silages, gkg^{-1} DM *(Đorđević et al., 2012a)*

Phenophase	Cut	Degree of wilting	pH	NH_3 N	Soluble N	Lactic acid	Acetic acid	Butyric acid
10% flowers	II	Low	4.84	188.24	732.18	64.38	57.94	0.00
		High	5.04	167.03	684.57	58.76	40.73	0.00
	IV	Low	4.86	153.95	715.29	56.45	48.30	0.00
		High	5.14	142.57	698.38	50.81	44.88	1.05
50% flowers	II	Low	4. 68	136.32	674.27	42.78	37.23	0.32
		High	4.75	108.62	660.32	40.31	42.61	0.00
	IV	Low	4.82	142.80	639.03	53.26	26.54	0.00
		High	4.95	113.37	617.48	47.03	25.42	0.00
Average for phenophase 10% flowers			4.97	162.95	707.60	57.60	47.96	0.26
Average for phenophase 50% flowers			4.80	125.28	647.78	45.84	32.95	0.08
Average for II cut			4.83	150.05	687.84	51.56	44.63	0.08
Average for IV cut			4.94	138.17	667.54	51.89	36.28	0.26
Average for low degree of wilting			4.80	155.33	690.19	54.22	42.50	0.08
Average for high degree of wilting			4. 97	132.90	665.19	49.23	38.41	0.26
Significance for phenophase			**	**	**	**	ns	ns
Significance for cut			**	**	ns	ns	ns	ns
Significance for degree of wilting			**	**	ns	**	ns	ns

ns - *no significance*; * ($p<0.05$); ** ($p<0.01$)

However, a material with a higher percentage of dry matter is difficult to compress. Residual oxygen in the silage mass enables more intensive and longer aerobic processes, and results in higher temperatures. In the experiment, *Đorđević et al. (2011b)* the significant effect of the degree of compaction on the share of ammonia nitrogen, soluble protein and true protein is determined. *Muck and Dickerson (1988)* studied the effects of different temperatures (15, 25 and 30°C) in ensiling alfalfa with 40 and 55% dry matter and found that proteolysis increases with temperature, but that temperature has a greater influence on proteolysis, compared to the percentage of dry matter.

Physical effectivity of the diet

The particle size of forage in the diet and physical effectivity (pe) of total mixed ration (TMR) may have significant influence on efficiency of utilization of dietary DM and CP in dairy cows. The minimal amount of physically effective fibre, which stimulates chewing activity, saliva production and reticulo-rumen contractions – is required in cow rations *(Stojanović et al., 2010; 2012)*. When NDF and peNDF in dairy cow rations is above minimally recommended values (25% NDF and 19% peNDF u SM, NRC, 2001), the decrease in particle size of lucerne haylage may improve the NDF digestibility in TMR, due to the increased surface exposed to microorganism activity and more intensive cellulolytic processes *(Stojanović et al., 2013; Yang and Beauchemin, 2006; Kononoff and Heinrichs, 2003).* The increase of fiber digestibility may have positive influence on CP digestibility, due to better utilization of ammonia nitrogen from degradable protein, which is used by cellulolytic bacteria for protein synthesis in the rumen. The positive effect from that is the increased amount of microbial protein synthesized in the rumen and likely higher availability of dietary protein to proteolytic enzymes in abomasum and small intestines due to smaller particle size *(Russell and Wilson, 1996).*

The opposite effect can be achieved by excessive decrease in particle size in feeds or TMR. For example, with the inclusion of ground lucerne hay instead of chopped, into TMR for dairy cows *Yang et al. (2002)* observed decrease in total chewing activity (and saliva secretion), which along with more intensive starch fermentation in the rumen brought to decrease of ruminal pH value. As a result there was a decrease in microbial N outflow into duodenum by 22% (Table 3). The efficiency of microbial synthesis (g microbial N/kg fermented OM in the rumen) was higher for the diet with chopped lucerne hay (26.9 g), compared to diet with ground hay (21.8 g). The shown difference suggests higher ruminal digestibility of chopped hay compared to the ground and also improvement in the intestinal N digestibility, which can be explained with the higher percent of microbial N in

rumen contents with chopped hay, while microbial N is more digestible than the N from feeds, which all amounted to higher protein digestibility. This example is a clear proof that physical properties of forages may have influence on their protein digestibility. The mild positive correlation (r=0.50) was observed between concentration of peNDF in the diet and total tract digestibility of NDF and N. There was also positive correlation between peNDF in the diet with microbial protein synthesis (r=0.49) and efficiency of protein synthesis (r=0.60). Authors conclude that concentration of peNDF in the diet can be a significant factor for predicting microbial synthesis and fiber digestibility in the rumen.

Table 3. The effect of different particle size in lucerne hay in TMR for dairy cows on intake and digestibility of nutrients *(Yang et al., 2002)*

Parameter	Haylage : Hay 50 : 50		Haylage : Hay 25 : 75	
	Chopped hay	Ground hay	Chopped hay	Ground hay
peNDF, % DM	26.7	20.9	24.3	19.7
NDF				
Intake, kg/day	8.56	7.83	7.29	6.82
Ruminal digestibility, % of intake	39.1	37.0	40.3	35.5
Postruminal digestibility, % of intake	14.4	9.6	11.3	7.7
Total digestibility, %	51.1	41.7	51.6	45.5
ADF				
Intake, kg/day	6.71	6.03	5.45	5.06
Ruminal digestibility, % of intake	45.2	40.5	41.3	39.1
Postruminal digestibility, % of intake	11.4	7.0	11.9	7.5
Total digestibility, %	54.2	45.3	53.2	46.5
N				
Intake, g/day	627.4	583.0	559.6	506.4
Duodenal inflow g/day				
Total N	607.7	541.3	546.7	473.6
Microbial N	273.8	226.2	272.3	198.7
Ruminal digestibility, % of intake	48.9	47.5	51.0	41.9
Postruminal digestibility, % of intake	63.3	56.1	61.9	60.2
Total digestibility, %	65.9	62.8	64.5	62.8

Conclusion

Although lucerne has high content and quality of protein, only a part of that potential is utilized in animal nutrition. The practical recommendations for optimal moment for lucerne cutting based on distinctive development phase are not very useful considering the rapid changes in its chemical composition, particularly in the first development phase. On the other side, new methods for evaluation of mean morphological stage were developed which enable very precise prediction of

nutritive value in lucerne (with plant classification and weighing the dry or fresh mass), which is the basic prerequisite for the maximal utilization of its potentials. The basic part of potential nutritive value is ratio of protein fractions, which are classified according to the rate of their degradation in the digestive tract. Some investigations confirmed that development phase has more influence on total crude protein content than on percentage of certain protein fraction within it. Also, it was confirmed that lucerne processing and conservation have high influence on protein fractions and content of degradable protein, which has effect on total protein digestibility and utilization. The biggest problem is occurring when lucerne is conserved as silage, because of the extensive proteolytic processed that occur. Those processes may be partly controlled with wilting and stimulation of lactic acid fermentation – with faster silage acidification. Also, the physical form of the diet has influence on protein utilization. The general conclusion can be that biological and physical availability of proteins (and their fractions) from lucerne is equally important as their content which can be a very variable parameter of its chemical composition.

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Proteinska vrednost lucerke u savremenoj ishrani životinja

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Rezime

U radu je, u vidu pregleda, prikazano više postupaka za utvrđivanje optimalne faze za korišćenje lucerke, što je glavni uslov za maksimalno korišćenje njene potencijalne hranljive vrednosti. Upotrebom srednje morfološke faze, nazvane Mean Stage by Weight (MSW), je moguće predvideti hranljivu vrednost lucerke sa visokom preciznošću. Složenost MSW metode je dovela do razvoja modifikovanog sistema predloženog od strane domaćih autora, nazvanog Mean Stage by Fresh Weight (MSFW), koji omogućuje jednostavniju upotrebu ovog sistema u praksi.

U više stranih i domaćih istraživanja utvrđeno je da faza razvića i ciklus vegetacije utiču u većoj meri na nivo ukupnih proteina, nego na međusobni odnos proteinskih frakcija, od čega realno zavisi stepen iskorišćavanja proteina. Brojni

rezultati ukazuju na činjenice da najveći značaj za odnos pojedinih proteinskih frakcija i stepen njihovog korišćenja imaju postupci konzervisanja i način korišćenja lucerke. U tom pogledu najveći problem se javlja kod silaže lucerke, za koju je neophodno preduzimanje odgovarajućih mera kontrole proteolitičkih procesa, koji mogu značajno da umanje iskorišćavanje proteina. Osim toga, upotreba različitih hraniva na bazi lucerke zahteva i adekvatno balansiranje obroka, kao i odgovarajuću fizičku formu, u skladu sa savremenim normativima i zahtevima.

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MANAGEMENT OF PERMANENT GRASSLANDS IN SERBIA: EVALUATION OF CURRENT FERTILIZER PRACTICE

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Abstract: The aim of this paper is to summarize some recent experiences and results in livestock feed production from permanent grasslands in Serbia with special emphasis being given to the importance of mineral fertilizer application and to the new role of manure enriched by zeolite. Also, trials assessed the benefits of mineral and organic fertilizers application in terms of forage production, testing whether the mineral or organic sources improves the stability of the grassland and evaluated response patterns over a large environmental gradient.

Key words: fertilizer, grassland, manure, quality, yield

Introduction

Grassland is the major resource to sustain the living of about one billion people worldwide. In industrialized Europe, grassland covers some 30 % of the agricultural area and forms the basis of a strong ruminant livestock sector (*Schnyder et al., 2010*). Beyond its contribution to meat and milk production, permanent grassland provides a number of environmental and social benefits. Grassland provides a broad range of services that are beneficial for man and plays a major role in, for example, the maintenance of biodiversity, carbon sequestration into soils, clean surface and ground water, and the provision of an attractive environment for recreation and leisure activities.

In the debate on climate change caused by anthropogenic greenhouse gas emissions, grassland is classified as an important carbon sink, due to higher organic matter contents compared to arable land use (*Osterburg et al., 2010*). Further, grassland constitutes a characteristic element of European cultural landscapes, and the maintenance of seminatural grassland habitats through traditional agricultural use is vital for the protection of biodiversity (*Zdanowicz et al., 2005*).

EU statistics distinguish permanent grassland, rough grazing, temporary grassland and various arable forage crops (e.g. green maize). In EU statistical

surveys, permanent grassland is defined as not being part of crop rotations for more than five consecutive years (*Osterburg et* al., 2010). It is used to grow herbaceous forage crops, either sown or natural (selfseeded), as pasture for grazing or for mowing in order to provide fresh forage for livestock kept indoors, or to produce hay or silage. Also, areas for rough grazing, e.g. semi-natural, low-yielding pastures, are permanent grassland. In contrast to permanent grassland, temporary grassland is part of arable crop rotations and thus it is seeded and regularly ploughed a few years later in order to establish other arable crops.

More than 80 % of the grazing livestock units in the EU-27 are cattle. Declining dairy cow numbers in EU have been the dominant trend since introduction of milk quota in 1984, which is often associated with losses of grassland area. In Central and Eastern Europe, grazing livestock herds diminished sharply in the 1990s during transition towards market economies (*Röder et al., 2007*).

Grassland farming in North-West Europe is changing. First, N-fertilization is decreasing, mainly due to legal restrictions (EU directive 91/676/EEC). Second, the importance of grazing in dairy farming is decreasing for different reasons, and consequently the importance of cut grass is increasing. Third, more dry summer spells are expected due to climate change (*Cougnon et al., 2014*).

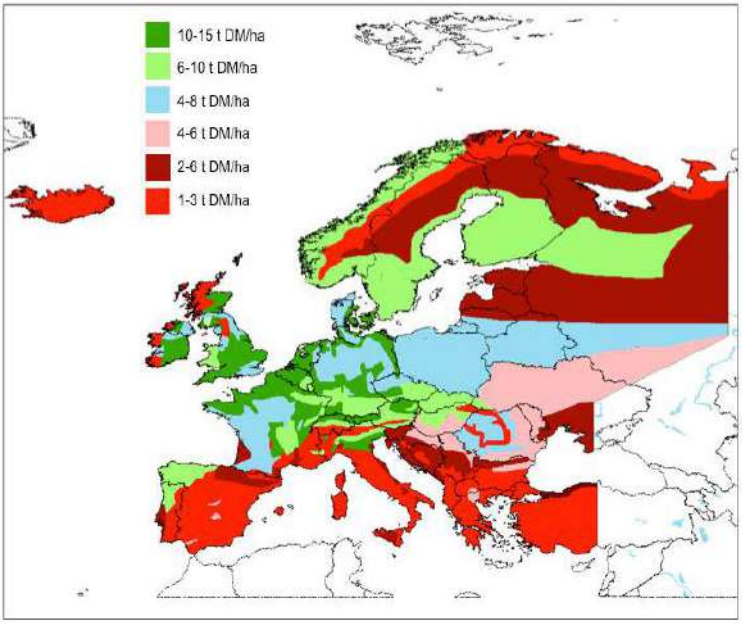

Figure 1. Production potential (annual yields in t DM ha^{-1}) of mown and heavily fertilized grasslands. (Source: *A. Peeters*, own calculations, cited by *Huyghe et al., 2014*).

Between 1975 and 2001, permanent grassland decreased by about 17 % in the EU-15; this is a rate of -0.7 % per year (*Gobin et al., 2006*). Apart from the loss of agricultural land due to urbanization, both conversion into arable land and afforestation or abandonment of farming contributed to this development.

Improvement of permanent grasslands in Serbia

Meadow vegetation of Serbia represents an outstanding resource for agriculture, occupying about 27% of the total agricultural area of the country (1.460.000 ha) as the most represented type of the agroecosystem (*Aćić et al., 2013*). Permanent grasslands, which serve both production and environmental purposes, are particularly important in hilly-mountainous areas of Serbia. On many farms they are the only source of forage. Main limitations in using of grassland resources is process of depopulation at the hilly-mountainous regions and migrating to urban centers and areas, which has been rapid during last decades (*Simić et al., 2013b*). Organized, systematic studies related to natural grasslands began in Serbia in the nineteen-fifties and intensified during the nineteen-sixties, which were characterized by strong economic development (*Stošić and Lazarević, 2009*).

The diminution of the productive potential of the permanent grasslands is being caused by erosion, to which unfavourable climatic conditions and unsuitable management may be added. Increased productive potential of these grasslands can be done by fertilization with different rates and types of organic and mineral fertilizers. The investigations carried out until now have shown the positive effects of manure, combined with moderate rates of mineral fertilizers, which are applied on grasslands. The permanent grasslands in Serbia generally situated on soils with low natural fertility, are of low productivity and have an unsuitable botanical composition. The main means for improving these grasslands consists of adjusting soil fertility, changing the dominance in the vegetation canopy and through good management. Two typical associations for permanent grasslands in Serbia are developed on the less productive soils. Stands of the ass. *Danthonietum alpinae* develop on the illuvial soil types, while the community *Asperulo-Agrostietum capillaris* develops on the brown soils and pseudoglay (*Aćić et al., 2013*). The most influencing factors determining development of these communities were the quantity of nutrients in the soil and the habitat moisture. Grassland vegetation of Serbia represents the habitat for range of many rare and endangered plant and animal species. Class *Festuco-Brometea* comprises 204 the Balkan endemic plant species, and the class *Molinio-Arrhenatheretea* 72 of such plants (*Šilc et al., 2014*). Negative influences, such as infrastructure building and habitat destruction, overgrazing, eutrophication, overharvesting of rare and endangered species,

cessation of traditional management and abandonment, led to qualitative and quantitative alterations of grasslands in Serbia. Balanced fertilization and rational use of fertilizers are basic measures for improving grassland. Organic fertilization and rational use of N fertilizers can produce substantial increases in the production and biodiversity, and in fodder quality improvement.

This paper presents the results obtained during longtime period (since 2000) on permanent grasslands, improved by fertilization with different rates and combinations of organic and mineral fertilizers.

Effect of fertilization on the yield

For improvement of production on grasslands the most important factor is mineral fertilizer. Experience with application of solid, good fermented manure is positive (*Simić et al., 2014, 2015a*); manure encourages the growth of legumes and high quality grasses, but manure available in hilly regions is primarily used on potatoes and cereals. Planning the quantities and type of fertilizer depends on the nutrient status of the soil and production potential of the grassland. Generally soils in Serbia, especially those under grass, are low in mineral nitrogen and total and available phosphorus, and have a medium or high quantity of available potassium (*Stošić and Lazarević, 2009*). Humification and mineralisation of nitrogen is low and slow because of low microbiological activity or marked acidity which are dominant characteristics of grassland soils, or because of drought in summer and cold in the winter.

Considering the floristic composition of grasslands (lack of legumes and good potential of grasses) the most important nutrient is nitrogen. Application date is determined by circumstances. Since grassland soils are on slopes, it is recommended and practiced that all fertilizers are applied at the same time in the spring, before the start of growth. For natural grasslands, especially 500 m above sea level, topdressing is not recommended for two reasons: firstly, their production is concentrated in the first half of the growing period, in the first cut; secondly, there is a dry period after the first cut and fertilizers still not dissolved are present on the soil for a longer period.

Nitrogen acquisition is one of the most important factors for plant production, and N contribution from biological N_2 fixation can reduce the need for industrial N fertilizers. N fertilization improves competitive abilities of grasses and increases productivity (*Simić et al., 2006*). Nitrogen application correlates strongly ($r>0.9$) and significantly ($P=0.01$) with dry matter yield (*Vučković et al., 2005c*). Therefore, it is necessary to reduce N fertilization to the detriment of yield but in favour of quality. Since Serbian grasslands have no potential for considerable

increase of legumes, N fertilizer has the decisive role, but in the presence of adequate P and K.

N-fertilization is decreasing in North-West Europe grassland farming, mainly due to legal restrictions (EU directive 91/676/EEC) (*Cougnon et al., 2014*). Research has shown that applying fertilizers on grasslands is economically justified since, generally, 1 kg of active element results in an increase of 80-100 kg of green matter (*Coman and Moisuc, 2011*). This economic productivity is also strengthened by the fact that 1 Mg ha^{-1} of dry matter can extract 20-21 kg of N, 6-8 kg of P, 20-21 kg of K and 10-14 kg of Ca (*Rotar et al., 2015*). The results showed that increasing dry matter yield (DM) does not necessarily mean a corresponding increase in sward fodder value quality. Small amounts of fertilizers can sometimes cause a large increase in quality as a result of the installation of mesotrophic species and a favourable substrate for *Fabaceae*.

Due to poor fertility of the soil and relatively severe climate, hilly-mountainous grasslands are often overgrown with plant species of low nutritional value. Most of them are eaten reluctantly or ignored by grazing animals. Yields of such grasslands might be improved by means of efficiently used fertilizers. Mountain grasslands respond to this treatment well and immediately (*Vučković et al., 2004, 2005a, 2007, 2010, 2014; Simić et al., 2015b*) showing changes in their plant composition and higher yields. With a view to producing higher yields, large quantities of N are often used. The maximum DM yield is recorded on the Sjenica-Pester plateau on a natural *Cynosuretum cristati* type meadow (1158 m asl) when 160 kg ha^{-1} nitrogen was applied and the yield amounted to 4.44 t ha^{-1} for two years (increase of 2.03 t ha^{-1} or 85.0% over the control). However, there is a question at what dose fertilizers would guarantee not only better grassland production, but also high quality of the grass.

Mineral fertilizer rates resulted in changes in the dominant species of grasslands, by increasing the percentage of high quality grasses Productivity increased, as well as forage quality compared to the unfertilized control. The trial carried out on *Agrostietum vulgare* - type meadow in the hilly mountainous region near Valjevo (at an altitude 750 m) showed the increase in the quantity of grass dry matter by application of N, P, and K fertilizers (*Vučković et al., 2007*). In particular, the increases in N rate had a favourable effect on DM yield. The maximum two-year average dry matter yield of 8.17 t ha^{-1} was achieved with the highest NPK rate (200:150:150 $kgha^{-1}$). The increase was 5.94 t ha^{-1} or 365% compared with the control. DM yield ratio between the highest NPK rate and control was similar in two consecutive years (indices 357 and 377, respectively). Similar result, but obtained on more productive meadow type *Arrhenatheretum elatioris* in the hilly mountainous region near Valjevo (altitude 450 m) showed maximum average dry matter yield of 7.97 t ha^{-1}, achieved also with the highest

NPK rate (200:150:150 kg ha^{-1}). The increase was 3.82 t ha^{-1} or 1.92-fold more compared with the control (*Ivaniš et al., 2013*).

Researches carried out on mountain Kopaonik on two *Danthonietum calycinae* meadows (1000 and 1500 m above sea level), confirmed that production of grassland depending on the fertilization, and cutting schedule (*Lazarević et al., 2009*). There were applied 3 fertilization treatments, with same amount of $N_{30}P_{30}K_{30}$ and additional spring nitrogen application (N_{30}, N_{50} and N_{70}), using 3 cutting schedules: forming of panicles, beginning of spike forming and full spike forming. Production of grassland and floristic composition demonstrated dynamic changes depending on the year, cutting schedule and applied fertilization treatments. The highest DM yield and crude protein production on both locations was obtained in late cutting, i.e. in the stage of full spike forming of dominant species and in fertilization treatment with $N_{80}P_{30}K_{30}$.

In the Vojvodina Province, permanent grasslands cover approximately 200,000 ha, of which area two thirds are pastures. Different soil types affect botanical composition i.e. herbage yield and quality of the permanent grasslands. Regardless grassland location, i.e., botanical composition and forage yield and quality, they receive no cultivation practices. Allomorphic soil types are common through the grasslands (at about 120,000 ha). They are concentrated in the region of Banat (80 %), where limeless solonetz is the predominant soil type. Forage yields obtained from grasslands of the Vojvodina Province are low (*Ćupina et al., 2005*). Even under rainfed conditions, appropriate agronomic practices ensure significant increases of herbage yield and quality. Generally, the climate in the Vojvodina Province, primarily in respect to the rainfall amount and distribution, is not suitable for high forage production. As usual, highest dry matter yields were obtained in the treatment with the highest N rate. On average, meadows produce one to two cuts per year.

Recent researches support using of zeolites as a binding agent for ammonia ions and as a soil additive on grasslands (*Simić et al., 2013a*). Application of the organic fertilizer enriched with zeolite on grasslands can be beneficial in achieving and maintaining a high output of herbage. Therefore, on small and middle-sized farms, where relatively small quantities of cattle manure are obtained every year, the organic fertilizer enriched with zeolite applied on grasslands may be used with good results (*Simić et al., 2014*). The obtained results show that the use of cattle manure enriched with natural zeolite can be used as a fertilizer for pastures which contributes to a preservation of nitrogen.

Legumes on permanent grasslands

Nitrogen limits the productivity of most terrestrial ecosystems including non-fertilised grassland. In the latter, biological N fixation is the main N input. Biological N fixation (BNF) is the main N input in unfertilised pastures and can provide transfer N to non-legume plants. This transfer can be coupled spatially or temporally to different degrees. In principle, three mechanisms are conceivable: (i) a direct transfer between living plants, (ii) a local but delayed transfer when non-legumes colonise a patch with decaying legume biomass, and (iii) a spatially diffuse transfer via the excreta of grazing animals. According to *Auerswald et al. (2010)* biological N fixation -N transfer occurred mainly via the excreta and was independent of the spatial distribution of legumes.

In southeastern Europe, the growth of legumes is seriously limited by the ability of each species to grow during usually cold winters (*Simić and Vučković, 2014*). There, the distribution of legumes on Serbian natural grasslands ranges from 6.73% to 34.12%, depending on plant nutrition (*Đurić et al., 2007*). The grasslands with a higher share of legumes and lower percentage of other plants are characterised by a higher crude protein content

Legumes present in Serbian grasslands are most often low producing species (*Trifolium alpestre, Trifolium montanum, Trifolium repens* with small leaves). If fertilization raises the share of legumes, yields are still low. The combination of PK will only contribute to an increase of the share of legumes if these are already present in the sward at 5 - 10% (*Stošić and Lazarević, 2007*).

In the study with undersown natural grassland on the Sjenica-Pešter plateau, the DM yield from the underseeded legumes (red clover and birdsfoot trefoil) is minimal in the humid vegetation season, but significantly higher over the control in the dry conditions (*Vučković et al., 2005a*). Underseeding has no positive effects on the chemical composition of forage.

Recent examinations of natural pastures in Serbia are focused to mark natural grasslands rich in annual legumes and determining species suitable for livestock. The emphasis is expected to shift from traditional selfregenerating species, such as alfalfa and clovers, to new species that are adapted to short periods, namely 1 or 2 years, of pasture which can be used in between extended cropping phases or phase pastures (*Simić and Vučković, 2014*).

Composition of permanent grassland

General characteristics of grasslands in Serbia are insufficient production and unsatisfactory quality. On areas that are not improved, weeds make over half

of the plant production. The considerable presence of weeds (forbs) in grasslands is due to insufficient quantities of fertilizer or unsuitable management. Only by combining these two measures can weeds be removed or eradicated. If mowing is carried out before seed-set of early annuals in the first year, they will not be present in subsequent years. With higher dosage of N plant composition became simplified mainly to high quality grasses, formerly seen as traces. Grassland could be changed by fertilization from ass. *Danthonietum calycinae* into ass. *Festucetum rubrae* on mountain Kopaonik (*Lazarević et al., 2009*). According to *Stošić et al., (2005),* there is regularity in changes to the floristic composition of grasslands: almost all associations transform into *Agrostietum vulgaris*, *Festucetum rubrae* or their transitional forms. A strong tendency towards the terminal stage of *Agrostietum vulgaris* was observed and registered when higher quantities of nitrogen were used.

In upland regions of the Vojvodina Province (the Fruška Gora Mountain), there are grasslands (meadows) with satisfactory botanical composition but poor yields (*Ćupina et al., 2005*). Investigations carried out on Stara Planina Mountain of 700–800 m a.s.l. (*Tomić et al., 2005*) identified two main plant associations: *Agrostietum vulgaris* with a total of 47 plant species and *Festucetum vallesiacae* with 77 plant species. High quality plant species present in these grasslands were classified into three categories: high quality grasses, high quality leguminous plants and useful or conditionally useful plant species belonging to other plant families. The association *Agrostietum vulgaris* had 11 useful grasses or 23.4%, 15 useful leguminous plants or 32% and 3 or 6.4% of useful and conditionally useful plants. The association *Festucetum vallesiacae* had 11 high quality grass species or 14.28%, 20 species of high quality leguminous plants or 26% and other useful and conditionally useful plants 11 or 14.28%.

In western Serbia, treatments with manure, organomineral fertilizer and mineral N application could change pasture composition in comparison to unfertilized pasture, which affected forage quality (*Simić et al., 2015a*).

Forage quality

Typical conservation of forage in the form of hay in Serbia can cause a decrease of crude protein (CP) content, because of improper handling, excessive drying or loss of leaves. Legumes are characterized by significantly higher CP content compared to grasses. Content of CP varied significantly in relation to crop and fertilization. Fertilizing with nitrogen and legume components positively affect the accumulation of crude protein in the sward (*Vučković et al., 2005a*), as well as ash and fat content, but a negative effect on cellulose content. Increased N rates increasing the contents of Cu, Co, and NO_3, and reduced the contents of K, P, Ca, Mg, S, Zn, and B (*Vučković et al., 2005b*). Nitrogen application correlates strongly

(r>0.9) and significantly (P=0.01) with chemical composition, positive correlation coefficients are between N application and crude protein, ash, fat (r=0.999**, 0.988**, 0.988**, 0.998**, respectively) (P=0.01), and negative correlation coefficient is between N application and crude fibre (cellulose), (r =-0.998**) (*Vučković et al., 2005c*). Also, correlation coefficient between dry matter and crude protein yield is highly significant in the Vojvodina province permanent grassland (*Ćupina et al., 2005*).

Recent researches with organomineral fertilizer application on pasture in western Serbia showed that usage of zeolite based fertilizer (zeolite enriched manure) lead to increase of true protein content and the decrease of non-protein nitrogen in grasses (*Simić et al., 2014*), what can have positive influence on forage digestibility.

Conclusion

The DM yield of permanent grasslands in Serbia is influenced very strongly by climatic conditions, type of grassland and level of organic and mineral fertilization. Results have shown the positive effects of fertilization on productivity, botanical composition and canopy structure of the studied permanent grasslands.

Beneficial effects of mineral fertilization on the yield of meadow swards should be seen also in terms of their environmental impact. Changes in species composition, water use and the density of the sod suggest that the application of fertilizers have effects which influence the development of sustainable multispecies swards with an acceptable yield and a good ability to retain rainwater.

Presented researches confirmed that permanent grasslands need to be maintained through mineral fertilizer application, especially application of nitrogen.

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Upravljanje permanentnim travnjacima u Srbiji: ocena aktuelnog načina đubrenja

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Rezime

Cilj ovog rada je predstavljanje novijih rezultata u proizvodnji kabaste krme sa permanentnih travnjaka Srbije sa posebnim osvrtom na važnost primene mineralnih đubriva, te na novu ulogu stajnjaka obogaćenog zeolitom. Takođe, procenjivana je dobit od primene mineralnih i organskih đubriva kroz proizvodnju krme, njenog prinosa i kvaliteta preko različitih nivoa ishrane, na velikom rasponu agroekoloških uslova proizvodnje.

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EFFECTS OF NITROGEN FERTILIZATION AND USING OF INOCULANT ON NUTRITIVE VALUE AND FERMENTATION CHARACTERISTICS OF WHOLE CROP MAIZE SILAGE

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Invited paper

Abstract: The aim of our study was to examine the effect of N fertilization and treatment of silo mass with bacterial-enzyme inoculant on chemical, fermentative and nutritional characteristics of whole maize plant silage, and to determine the correlation between certain quality parameters. The experiment was carried out on experimental field and in the experimental laboratory of the Institute for Animal Husbandry. Corn was planted in the experimental field in four repetitions. The effect of four different nitrogen rates of 0, 60, 120, 180 kg N ha^{-1}, add to the corn crops during the growing season was studied. The silage was prepared in the laboratory and stored in vacuum bags. One part of the chopped mass was ensiled using the bacterial-enzyme inoculant, and the other partn without inoculant. Silage was stored at room temperature for 90 days. After 90 days, silage was sampled for chemical analysis. Fertilization with nitrogen in the amount of 120 kg N ha^{-1} resulted in an increased content of CP, CFT. N fertilization had a positive impact on the content of ME and NEL. The highest content of ME and NEL were determined in treatments with 120 and 180 kgN ha^{-1} of 10.3 MJ kg^{-1} ME and 6.0 MJ kg^{-1} NEL. The treatment with bacterial-inoculants considerably reduced the content of CP, NH_3-N, acetic acid, the pH value and increased the proportion of lactic acid relative to the acetic LA/AA.

Key words: nitrogen, bacterial-enzyme inoculant, whole crop maize silage

Introduction

In the intensive agricultural production and ruminant nutrition corn silage is very valuable voluminous energy feed. It is represented in livestock production throughout Europe and America. In Serbia it dominates in ruminant nutrition for

many years. Compared to other forage silages, silage from whole maize plant has high energy content in dry matter, high palatability, high sugar content and easier technological procedure of preparation *(Forouzmand et al., 2005).*

For optimum production of maize it is necessary to add large amounts of N in the form of nitrogen fertilizers. Supplemental fertilization with N significantly affects the yield increase. *Amanullah (2010),* in examination of the effects of different levels of nitrogen fertilization on the biomass yield concluds that the biomass yield significantly increases with the addition of N in an amount of 150-200 kg ha^{-1}. According to research by *Zhang et al. (2015)* fertilization of corn with N in amounts of 0, 79, 147, 215 and 375 kg N ha^{-1}resulted in the increase of average yield of fertilized treatments from 17 to 20%, except in case of > 215 kg N ha^{-1}ofnitrogen which did not lead to increasing yields. In addition to the yield, N fertilization affects the chemical composition of green mass (fresh forage). In studies of *Shaeffer et al. (2006),* nitrogen fertilization shows little effect on forage quality variables except for CP concentration. However, studies of *Islam et al. (2012*) show nitrogen fertilization not only increasing the crude protein content, but also the dry matter content, metabolizable energy and in vitro dry matter digestibility and reducing the content of the ADF. As for the easily soluble carbohydrates (WSC) as the primary substrate for the growth and development of fermentable lactic acid bacteria, some studies suggest that their content in plants is reduced by adding N fertilizer (*Almodares et al., 2009),* while some studies report their increase *(Islam et al., 2012).*

In order to achieve a good quality silage it is necessary in the initial stages of fermentation to achieve a rapid reduction of the pH, to avoid the occurrence and growth of harmful microorganisms, to avoid losses of dry matter and increase aerobic stability of silage. This can be achieved by the addition of bacterial and bacterial-enzyme inoculants *(Jatkauskas et al., 2012).* These supplements will lead to reduced fiber content, increasing the concentration of sugar and lactic acid and increasing the digestibility of silage (*Đorđević et al., 2011*). In addition, microbial additives act on animal performance by increasing the nutritional value of the silage, and some strains of LAB can survive in the gastric juice (rumen fluid) and play the role of buffer thus maintaining the activity of a cellulase enzyme and thereby increasing the digestibility (*Weinberg et al., 2003*), and therefore the consumption and animal performance *(Ando et al., 2006).*

The dry matter content in plants must be adequate, because in the silo mass which has a higher dry matter content, the effect of inoculants is smaller, i.e. it decreases with increasing dry matter content (*Comino et al., 2014*). Optimum value of DM should be around 35% when the best balance is achieved between starch as

a carrier of energy value and soluble sugars needed to produce enough lactic acid and lowering the pH (*Horrocks and Vallentine, 1999*).
The aim of our study was to examine the effect of N fertilization and treatment of silo mass with bacterial-enzyme inoculant on chemical, fermentative and nutritional characteristics of whole maize plant silage, and determine the correlation between certain quality parameters.

Materials and Methods

Maize hybrid (NS 6030 and ZP 666) was planted on 12th April 2013 at a planting rate of 64,900 plants ha^{-1} at the experimental field of the Instutute for Animal Husbandry, Belgrade, Serbia (44° 49′ 10″ N, 20° 18′ 45″ E). The experimental field was divided into four blocks to obtain four replication per treatment and each block was split into four plots for the four treatment of nitrogen fertilization (0, 60, 120, 180 kgN ha^{-1}). Fertilization was done on 15th May 2013. with mineral fertilizer KAN (27%N). Maize was harvested in August, as whole-crop (cutting hight 20 cm) and chopped to a theoretical cut lenght of 10 mm. From each plot chopped mass was divided on two parts. One part was untreated and second treated with bacterial-enzyme inoculant Sill-All 4x4+WS (*Lactobacillus plantarum, Pediococcus acidilactici, Pediococcus pentosaceus, Propion bacterium acidi propionici*, α-amilaza, celuloza, beta-glukanaza, ksilanaza). The inoculant was applied at recomended rate of 5g t^{-1} fresh maize material. The chopped forages from each plot was than ensiled in the 200 x 300 x 0.9 cm vacuum plastic bags with package mashine. Before filling of plastic bags, fresh maize forage samples were taken for chemical anallisys (Table 1.) The silages were conserved at room temperature for 90 days. After period od 90 days silages were opened and contents were sampled to determine the DM content, chemical and fermentable composition.

Table 1. The chemical composition of maize forages at ensiling

level of fertilization	0	60	120	180
DM (g kg^{-1})	431.3	472.9	448.4	477.4
CP (g kg^{-1} DM)	71.6	78.6	81.4	104.7
NDF (g kg^{-1} DM)	496.8	495.2	552.7	510.7
ADF (g kg^{-1} DM)	260.4	268.1	283.2	266.5
WSC (g kg^{-1} DM)	40.2	49.5	23.6	19.0
BC (Meq/100 gDM)	42.7	39.3	41.5	40.2

DM-dry matter; CP-crude protein; NDF-neutral detergent fiber; ADF- acid detergent fiber; WSC-water soluble carbohydrates; BC-buffering capacity

Chemical analysis

Dry matter content was determined by drying the samples at 105°C overnight. Crude protein content was determined according to Kjeldahl *(AOAC 1990)*, content of crude fat using the Soxhlet method and ash by heating the dry samples in anoven at 550°C for 2h. Neutral detergent fiber (NDF) and acid detergent fibre were analysed according to Van Soest method. Water soluble carbohydrate (WSC) content was determined using the method of Luff-Schoorl. Ammonia nitrogen was determined by the distillation method using a Kjeltec 1026 analyser and the pH value was measured with a Hanna Instruments HI 83141 pH meter. Lactic acid (LA) and volatile fatty acids [acetic (AA) and butyric acid (BA)] were quantified by a gas chromatographic system (GC-2014, Shimadzu, Kyoto, Japan) equipped with flame-ionization detector and auto sampler and injection system, using a NukolTM (30m × 0.53mm × 0.5µm) capillary column (Supelco, Sigma-Aldrich Co.) *(Faithfull 2002).* Total digestible nutrients value (TDN), relative feed value (RFV) and values of metabolic energy (ME) and net energy for lactation (NEL) were calculated using the following steps:

TDN (%)= (-1,291 x ADF) + 101.35
RFV (%) = DDM (%) x DMI (%) x 0.775
DDM (%) = Digestible Dry Matter = 88.9 - (0.779 x % ADF)
DMI (%) = Dry Matter Intake = 120 / (% NDF)
Calculation of TDN and RFV was done according to *Harrocks and Vallentine (1999).*
ME(MJ kg^{-1}) = (0.01715 x dCP) + (0.03766 x dCf) + (0.0138 x dCF) + (0.01464 x dNFE)
NEL(MJ kg^{-1}) = ME x kl
kl = 0.6 x (1+ 0.004 x (q-57)) x 0.9752
q (%) = (ME / GE) x 100
GE(MJ kg^{-1}) = (0.02414 x CP) + (0.03657 x Cf) + (0.02092 x CF) + (0.01699 x NFE)
CP - crude protein (g kg^{-1});
Cf - crude fat (g kg^{-1});
CF – crude fibre (g kg^{-1});
NFE – nitrogen free extracts (g kg^{-1});
d – digestible
q - metabolizability coefficient

The experimental data ME and NEL were calculated by formula according *Obračević (1990).*

Statistical analysis

The experimental data were processed by the method of analysis of variance (two factor experimental design), applying the programme ANOVA and means were compared using t test.

Results and Discussion

The chemical composition and energetic characteristics of treated and untreated whole maize plant silage is shown in Table 2. Fertilization treatments had a significant impact on the content of crude protein, crude fat, metabolic energy and net energy for lactation. Nitrogen fertilization led to an increase in crude protein content. However, this increase was determined only in the treatment with 120 kgN ha^{-1}. Nitrogen fertilization that is > 120 kgN ha^{-1} reduced the crude protein content in the silage. The content of crude fat also increased by the addition of N mineral fertilizer up to 120 kgN ha^{-1}. Further adding of nitrogen led to a reduction of crude fat in the silage. N fertilization had a positive impact on the content of ME and NEL. The highest content of ME and NEL were determined in treatments with 120 and 180 kgN $ha^{-1}$of 10.3 MJ kg^{-1} ME and 6.0 MJ kg^{-1} NEL.

Table 2. Nutritional (g kg^{-1} DM) and energetic characteristics (MJ kg^{-1}) of maize silage after 90 days of conservation

Lev. of fertil.	0		60		120		180		F effect	I effect	FxI effect
Treatm	C	T	C	T	C	T	C	T	P value		
DM	423.7	430.3	463.5	469.4	446.4	440.6	474.3	467.0	0.235	0.906	0.302
Ash	28.5	30.4	27.8	27.3	31.4	29.6	29.3	31.0	0.061	0.705	0.344
CP	55.4	48.1	58.6	53.9	80.1	71.8	79.4	76.2	<0.001	0.036	0.906
CFT	23.8	23.5	25.0	19.3	24.5	25.3	20.8	18.4	0.003	0.059	0.120
NDF	504.6	537.6	503.8	508.6	510.9	491.8	477.1	532.1	0.567	0.089	0.088
ADF	281.8	279.7	279.7	276.0	291.6	281.6	250.3	278.2	0.161	0.671	0.245
TDN%	66.9	66.5	67.2	63.9	66.2	68.2	66.5	65.1	0.524	0.386	0.219
RFV%	132.4	121.9	134.2	115.7	133.9	137.8	124.2	119.0	0.080	0.060	0.242
ME	10.2	10.2	10.0	10.2	10.3	10.2	10.3	10.3	0.007	0.915	0.221
NEL	5.9	5.9	5.8	5.9	6.0	5.9	6.0	5.9	0.033	0.801	0.189

C-untreated; T-treatment with inoculant; F-effect of fertilization; I-effect of inoculation; FxI- effect of interaction; DM-dry matter; CP-crude protein; CFT- crude fat; NDF-neutral detergent fibre; ADF- acid detergent fibre; TDN-total digestible nutrient; RFV-relative feed value; ME-metabolizable energy; NEL-net energy for lactation

In the research by *Islam et al. (2012)* N fertilizer has also led to a significant increase in the content of crude protein and metabolizable energy in the silage. Increasing the content of CP in the treatments with fertilization can be explained primarily by increasing the crude protein in the plant before ensiling, while the increase in ME is explained by the increasing content of CP, and reduction in ADF and NDF *(Asgharzadeh et al. 2013).*

A significant effect of adding the inoculant was reflected only in the content of crude protein. Namely, the addition of lactic acid bacteria and enzymes to the silo mass led to a reduction in the concentration of crude protein in the silage. Also, in the research of *Ruiz-Perez et al. (2012)* and *Dupon et al. (2012),* crude protein content decreased with the addition of the bacterial inoculant, while in the study by *Vakily et al. (2011)* it was uniform.

The other investigated parameters shown in table 2 had not significantly changed in relation to the studied factors. According to *NRC (2001),* corn silage with over 40% dry matter contains 65.4% TDN, 44.5% NDF, 27.5% ADF and 4.0% ash. The values obtained were similar to TDN values of *NRC (2001),* and the values for NDF and ADF slightly higher, indicating that the silage was prepared at a later stage of maturity.

In table 3 are presented fermentation end product and the pH value of maize silage after 90 days of conservation. N fertilization had a significant impact on fermentable features of the silage, however, in certain tested parameters, such as lactic acid, the differences depending on the level of N fertilization can be observed. The treatments with 60 and 120 kgN ha^{-1} had higher lactic acid content compared to the control. This is confirmed by the research of *Namihira et al. (2010)* who has concluded that N fertilization is important silage quality factor and significantly increases the content of lactic acid to the fertilization level of 150 kg N ha^{-1}.

Table 3. Fermentation end product (g kg^{-1} DM) and pH value of maize silage after 90 days of conservation

Level of fertilization	0		60		120		180		F effect	I effect	FxI effect
Treatment	C	T	C	T	C	T	C	T	P value		
NH_3-N†	51.6	47.7	58.9	50.1	68.2	44.2	64.3	50.1	0.155	0.002	0.033
Lactic acid	69.2	67.6	76.6	72.4	72.7	72.2	68.1	67.4	0.181	0.311	0.593
Acetic acid	12.2	7.9	13.9	9.7	13.5	12.3	12.4	8.8	0.210	0.001	0.674
LA/AA	5.9	8.9	6.4	7.9	5.4	6.2	5.6	8.2	0.064	<0.001	0.247
Butyric acid	0.4	0.4	0.6	0.4	0.8	0.6	0.6	0.3	0.315	0.202	0.840
pH	3.9	3.8	3.9	3.8	4.0	3.8	3.9	3.8	0.363	<0.001	0.889

†-(g kg^{-1} TN); C-untreated; T-treatment with inoculant; F-effect of fertilization; I-effect of inoculation; FxI- effect of interaction; LA/AA-lactic acid/acetic acid

Treatment with bacterial-enzyme inoculants had a significant impact on the content of ammonia nitrogen, acetic acid, pH value and LA/AA. Silages treated with inoculants showed a lower content of ammonia nitrogen and according to its content, which is less than the limit 7-10% (*Đorđević and Dinić, 2003*), they are classified as high quality silages. Also, the content of acetic acid and pH were

lower in silages with inoculant. *Rota et al. (2012)* in their research also report lower content of acetic acid and pH in silages treated with *Pediococcus pentosaceus* and *Lactobacillus plantarum*. However, in recent years, the opinion prevailed that the acetic acid, particularly acid created during the anaerobic phase of lactic acid, is essential for the preservation of aerobic stability of silage. The hetero-fermentative bacteria that are integral part of some bacterial inoculants sare responsible for this. For this reason, *Comino et al. (2014)* reported a higher content of acetic acid in the silage with inoculant as the inoculant used, in addition to homo- also had hetero-fermentative bacteria such as *Lactobacillus buchner*. Otherwise, the content of acetic acid from a control treatment is similar to the content of the present study. The level of acetic acid in the investigated silages was satisfactory (<3-4%) and the ratio LA/AA (>3:1), indicating a good fermentation in the control and the treatment with inoculant.

According to *Đorđević and Dinić (2003),* pH value of silage should be in the range from 3.8 to 4.5. If the pH is lower than 3.8 it is less consumed. In our research the pH value of silage from the control and treatment was 3.8-4.0.

The results of correlation coefficients between tested chemical and fermentable parameters (Table 4) showed a significant negative correlation between the dry matter content in silage and content of lactic, acetic, butyric acid, TDN and RFV. Also, negative correlation was established between the ash content and the ADF with TDN and RFV.

Table 4. Correlation analysis of chemical and fermentable parameters of the silage

	NH_3-N	pH	LA	AA	BA	TDN	RFV	ME	NEL
DM	-0.08	-0.28	-0.49*	-0.54*	-0.33*	-0.06	-0.36*	0.17	0.21
Ash	-0.13	-0.14	-0.02	0.03	-0.03	-0.44*	-0.32*	0.17	0.10
CP	0.22	0.19	0.21	0.41*	0.31*	0.41*	0.19	0.40*	0.28
CFT	-0.10	0.40*	0.24	0.38	0.31	-0.06	-0.02	-0.14	-0.12
NDF	-0.08	0.05	-0.01	-0.04	0.10	-0.53*	0.31*	-0.12	-0.20
ADF	-0.02	0.07	-0.18	-0.10	-0.00	-0.97*	-0.60*	-0.19	-0.26

*- Marked correlations are significant at p <0.05; DM-dry matter; CP-crude protein; CFT- crude fat; NDF-neutral detergent fibre; ADF- acid detergent fibre; TDN-total digestible nutrient; RFV-relative feed value; ME- metabolizable energy; NEL-net energy for lactation; LA- Lactic acid; AA- acetic acid; BA-butyric acid

Increase of the NDF content decreased TDN and increased RFV. The crude protein content was significantly positively correlated with certain fermentable parameters. Thus, increase in the crude protein content in the silage increased the content of acetic, butyric acid, TDN, RFV and ME: r=0.41, r=0.31 and r=0.41, r=0.40 respectively.

With maturation of plants the dry matter content increased, and therefore the NDF and ADF, causing the decline in crude protein and metabolic energy. Such high

fibre and low protein concentrations decrease overall digestibility and RFV (*Asgharzadeh et al., 2014*).

Conclusion

On the basis of the examined factors of the quality and nutritive value of silage from whole maize plant, we concluded that nitrogen fertilizer contributed favourably to the quality and energy value of silage, but only to the level of nitrogen of 120 kgN ha^{-1}. Further adding of nitrogen had a depressive character. N fertilization led to a significant increase in crude protein, metabolizable energy and net energy for lactation. It also led to a minimum increase in the content of lactic acid.
Application of inoculants had a significant impact on the improvement of the quality of the fermentation, particularly in the reduction of the content of ammonium nitrogen, acetic acid and the pH. However, the silages from control treatments also had satisfactory investigated parameters of quality and nutritional value so that both can be classified as good quality silage.

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Efekat đubrenja azotom i inokulanta na hranljivu vrednost i fermentabilne karakteristike silaže od cele biljke kukuruza

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Rezime

Cilj naših istraživanja je da ispitamo efekat đubrenja N i tretmana silo mase bakterijsko-enzimskim inokulantom na hemijske, fermentativne i nutritivne karakteristike silaže od cele biljke kukuruza, kao i da utvrdimo korelacionu zavisnost pojedinih parametara kvaliteta. Ogled je izveden na oglednom polju i u eksperimentalnoj laboratoriji Instituta za stočarstvo. Kukuruz je posejan na oglednom polju u četiri ponvljanja. Ispitivan je uticaj četiri različite doze azota od 0, 60, 120, 180 kgN ha^{-1}, dodate u toku vegetacije kukuruza. Silaža je pripremana

u laboratoriji. Silaža je pripremana u vakum kesama. Jedan deo iseckane mase siliran je sa bakterijsko-enzimskim inokulantom, a deo bez dodatog inokulanta. Silaža je čuvana na sobnoj temperaturi 90 dana. Nakon devedeset dana silaža je uzorkovana za hemijske analize. Đubrenje sa količinama azota do 120 kgN ha^{-1} dovelo je do povećanja sadržaja CP, CFT. Đubrenje azotom imalo je pozitivan uticaj na sadržaj ME i NEL. Najvećim sadržajem ME i NEL odlikuju se tretmani sa 120 i 180 kgN ha^{-1} od 10.3 MJ kg^{-1} ME i 6.0 MJ kg^{-1} NEL. Tretman bakterijsko-enzimskim inokulantima je značajno smanjio sadržaj CP, NH_3-N, sićetne kiseline, pH vrednost i povećao udeo mlečne kiseline u odnosu na sirćetnu LA/AA.

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EFFECT OF WATER STRESS ON SOYBEAN PRODUCTION

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Abstract:

Soybean is main source of vegetable protein and oil in the world. Soybean used for livestock feed, human food and industrial processing. Soybean is main raw material for the preparation of a protein feed for all types and categories domestic animals. In the world the largest part of soybean produced, about 70% is used to feed livestock. In Serbia the area under soybeans and grain yield varies from 144386 ha to 170255 ha and 1.73 t ha^{-1} and 3.18 t ha^{-1}, respectively. Grain yield depends on the amount of rainfall from late June to early September when the highest needs for water (since beginning of flowering until the end of grain filling). Soybean is most sensitive to drought stress during the pod formation and grain filling stages.

Key words: drought, soybean, area, production, yield

Introduction

The economic and agro-technical importance of soybean (*Glycine max* (L.) Merr.) is very large in Serbia and the world. It is a multipurpose crop, ideal for livestock feed, for human food, and industrial processing. Soy protein is the best plant proteins. The great economic importance of soybean reflected in the high nutritional value of grain. Commercial varieties of soybean in grains contain an average 40% protein, 20% oil, 35% carbohydrate, and 5% ash on a dry-weight basis *(Liu, 1997)*. In world, livestock industry is the largest consumer of soybean meal as a byproduct in processing. Thus, in the U.S.A. 98% soybean meal goes to feed cows, pigs and chickens *(Soyatech, 2014)*. Soybeans can be used as soybean meal, cake and a green mass, hay and silage for livestock feeding. For the production of green mass, soybean can be grown as a pure crop or in mixtures with other plants (maize, sorghum, sunflower). When grown as pure crop, it can give

30-50 t ha^{-1} of green mass, which provides 1000 to 1500 kg of high-quality protein and 800 to 1000 kg of mineral substances. Soybean produces a high yield of green mass in intercropping (25-40 t ha^{-1}). However soybean grain contains a complex of antinutritive substances (trypsin inhibitors, hemagglutinin, saponin, goitrogens factors, allergens, flatulene substances lipoksidaza, urease) which is why it must be heat-treated before use. Therefore, the selection of soybean varieties is focused on creation of Kunitz trypsin inhibitor (KTI) free varieties in the mature grain (*Srebrić, 2013*). KTI free varieties in the mature grain can be used in the diet of adult non-ruminant animals without heat treatment thereby reducing costs of production of animal feed (*Ranđelović et al., 2004; Ranđelović et al., 2006)*. In the industrial processing, grain of these varieties is processed at a lower temperature and for a shorter time, reducing processing costs, while beneficial proteins are denatured to a lesser degree (*Ranđelović, 2009*). In the soybean production in Serbia two Kunitz trypsin inhibitor free varieties of soybean are implemented - Laura (I maturity group) and Lana (maturity group II) that do not require specific growing conditions in relation to the varieties of standard grain quality. Results by *Ranđelović et al. (2006), Ranđelović (2009)* and *Ranđelović et al. (2010)* showed that cultivar Lana had higher grain yield then cultivar Laura. *Ranđelović et al. (2009)* concluded that the cultivar Lana was more productive in climatically favorable year, while variety is Laura, with shorter vegetation period, in arid year. In order for raw soybean to be used in the diet of young domestic non-ruminant animals, it has to be free of KTI and lectins *(Palacios 2004)*. In Serbia, soybean it is a major ingredient in livestock feed, very little used in the human food and industrial processing *(Ranđelović, 2009)*. Soybean seeds used as a supplement to cereal seeds in feed dairy cows, cattle, pigs, goats, sheep, horses and poultry *(Iqbal et al., 2003; Ranđelović, 2009)*. The soybean meal and soybean flour used in the nutrition of livestock, poultry and fish *(Popović et al., 2009, 2011, 2013)*. *Todorović and Kondić (1993)* concluded that the one kilogram of soybean flour is equivalent to 2.3 kg of meat and 12 litres of milk. The soybean flour is used in the baking industry. Soybeans represent 50 percent of world oilseed production. The soybean oil used in food industry, while lecithin (component of soybean oil) apart from being used in food industry for production bakery and confectionery products, has its application in medicine, textile and chemical industries. Soybean oil is used for biodiesel production.

The agro-technical importance of soybean reflected in crop rotation, because enriches the land with nitrogen. Nitrate-fixing symbiotic bacteria *Bradyrhizobium japonicum* on soybean root convert inorganic nitrogen N_2 from the atmosphere into a form NH_2 (form appropriate for the plant). *Bethlenfalvay et al. (1990)* reported that the soybean-*Bradyrhizobium* symbiosis can fix from 40 to 300 kg N ha^{-1} per year, which is equivalent to 500-1000 kg ha^{-1} of mineral nitrogen

fertilizers. Also, soybean it improves the physical and chemical properties of the soil. Soybean his powerful developed and deep root system has a positive influence on physical (structure) and chemical properties (fertility) of the soil. Root secretions have a positive influence on maintaining and improving fertility in soil because activated the nutrient mobility and nutrient mineralization (i. e., the breakdown of nutrients into plant usable forms) *(Hrustić et al., 1998).*

World, USA and Serbia soybean production

The total harvested area under soybean in the world for the 2009-2013 periods was 104.5 million ha, total production 253.5 million tons and average grain yield 2.43 t ha^{-1} *(FAOSTAT, 2014)*, Table 1.

Table 1. Harvested area, production and grain yield in soybean in world, USA and Serbia, 2009-2013 *(FAOSTAT, 2014)*

Year	Harvested area, ha			Production, t			Grain yield, t ha^{-1}		
	World	USA	Serbia	World	USA	Serbia	World	USA	Serbia
2009	99337807.58	30906980	144386	223411328.80	91417300	349193	2.25	2.96	2.42
2010	102807828.03	31003300	170255	265120391.98	90605460	540859	2.58	2.92	3.18
2011	103816640.88	29856410	165253	261886302.22	84191930	440847	2.52	2.82	2.67
2012	105018859.07	30798530	162714	240971129.75	82054800	280638	2.30	2.66	1.73
2013	111544703.08	30703000	159724	276032361.62	89483000	385214	2.48	2.91	2.41
M	104505167.73	30653644	160466	253484303.87	87550498	399350	2.43	2.85	2.48
Index, %	100.00	29.33	0.15	100.00	34.54	0.16	100.00	117.28	101.02

Soybean is grown mainly from 0° to 55° latitude and below to 2000 m elevation, but most commercial production is between 25° to 45° latitude and below 1000 m elevation *(Fageria et al., 2010).* The USA, Brazil, Argentina, China and India are the largest producers of soybeans, yielding more 92% of the total world production. The European largest soybean producers are the Russian Federation, Ukraine, Italy, Republic of Serbia and Romania *(Vlahović et al., 2013).*

Harvested area, total production and grain yield of soybean in the Republic of Serbia for the 2009-2013 periods was the 160466 ha, 399350 tons and 2.48 t ha^{-1}, respectively. Harvested area, total production and grain yield of soybean in Serbia vary among years in range from 144386 ha (2009) to 170255 ha (2010), 280638 t (2012) to 540859 t (2010) and 1.73 t ha^{-1} (2012) to 3.18 t ha^{-1} (2010), respectively. Unstable and reduced soybean yields in Serbia are the result of insufficient amount and irregular distribution of rainfall during the growing season, especially from late June to early September (from the beginning of flowering until the end of grain filling). *Mandić et al. (2013)* reported that in Serbia the variation of rainfall regime is typical during summer seasons. *Srebrić and Perić (2014)* state

that the extremely dry summer of 2012 led to a significant reduction grain yield, while *Đukić et al. (2014*) reported that 2010 was the favorable year for soybean production. The five-year average grain yield of soybean in Serbia is higher for 1.02% (0.05 t ha^{-1}) than average grain yield of soybean in world, but lower for 87.02% (0.37 t ha^{-1}) than average grain yield of soybean in USA. Introduction of irrigation would enable the achievement of high and stable yields of soybean and the changing climatic conditions in Serbia. However, in Serbia are irrigated about 1% of arable land *(Mandić et al., 2013)*.

Effect of water stress on soybean production

The soybean water requirement in Serbia is 450-480 mm during the growing season *(Glamočlija, 2012)*. Monthly needs of soybeans for water are the following: 10 to 40 mm in April, 30 to 60 in May mm, 90-110 mm in June, 100 to 125 mm in July, 100 to 120 mm in August, 50 to 80 mm in September and 40 mm in October. The highest needs for water are since beginning of flowering until the end of grain filling, when soybean crop consumes 60-90% of total water needed. This, depending on the maturity group, refers to the period from late June to early September *(Srebrić and Perić, 2014)*. *Dolijanović et al. (2013)* reported that 1 mm of annual rainfall increase the soybean grain yield from 2.1 to 2.9 kg ha^{-1}. Drought and high temperatures during flowering and grain filling stages causes abortion of flowers, reduced pod number, number of seeds per pod, and grain remains small *(Ranđelović, 2009).* Commercial varieties developed in Serbia have stem of indeterminate growing type which means that on the same plant basically formed pods appear, in the central part the open flowers and on the top of the stem new leaves are still emerging. During growth, stem flowering takes place successively from the lower nodes to the top and lasts 3-4 weeks. Long flowering compensates rejection/abortion of flowers and pods that occurs in case of drought. The plant rejects flowers or pods already formed in the lower part, while the top of the stem emphasizes the formation of new flowers. The level of compensation is gradually reduced with the end of flowering, and completely ceases with the completion of the formation of flowers, which is in the mid-stage of grain formation. This is why the worst effects of drought are in the mid-stage of the formation of the pods to the mid-stage of grain formation. In addition, soy begets more flowers per plant than it has the potential to feed the pods, usually only 25-30% of flower conceived are formed. Soybean yield may be reduced if during the grain filling stage, which lasts until the mid-stage of full grain formation, a drought occurs. The negative consequences of long flowering is uneven ripening which on one hand makes it difficult to harvest, and on the other hand increases the risk of seed loss, as the ripe pods shatter easily at harvest. During flowering and pod set temperatures above

32°C leads to abortion of flowers and decline of pods (*Glamočlija, 2012*). High temperatures and drought in the summer months lead to faster maturing of soybean crops with shortening of flowering and grain filling stages. Unfavorable climatic conditions reduce soybean grain yield by 24% to 54% (*Kobraee and Shamsi, 2012*). *Bošnjak (2004)* states that the grain yield of soybean is highly significantly correlated with the amount of rainfall in the growing season and with the amount of rainfall in June, July and August, when the soybean is flowering, pod formation and grain filling stages. Soybean yield significantly depends on the amount of rainfall in July and early August *(Vidić et al., 2009, Popović et al, 2015)*. Grain yield and yield components of soybean declined in drought stress *(Ranđelović et al., 2010; Kobraei et al., 2011; Mandić et al., 2015)*. *Mandić et al. (2015)* reported that quantitative traits of soybean (plant height, first pod height, number of nodes per plant, number of pods per plant, number of grain per plant, grain yield per plant, 1000-grain weight and grain yield) have the higher values in year with favorable distribution of rainfall. These authors reported that the drought stress in August (stage of grain filling) leads to a decrease in soybean grain yield (Figure 1). Also, *Ranđelović (2009)* and *Ranđelović et al. (2010)* concluded that the amount of rainfall in August has a decisive influence on soybean yield.

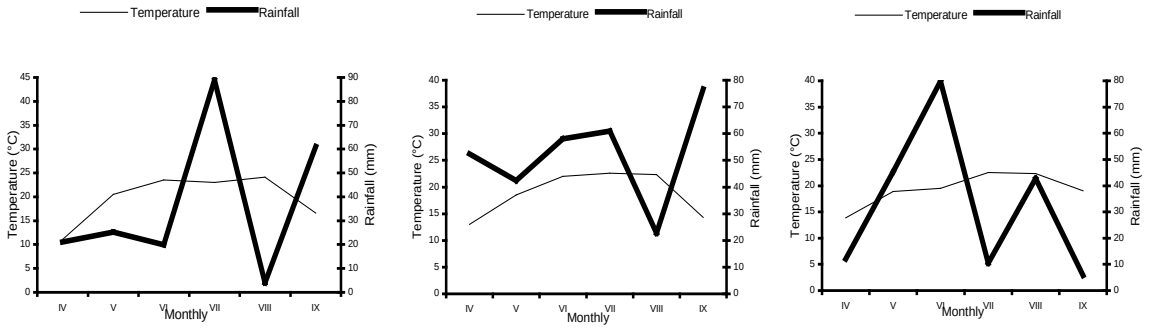

Figure 1. Climate diagram according to Walter in the 2003 for Ivanovo *(Ranđelović, 2009)*, 2008 for Putinci *(Mandić et al., 2015)* and 2009 for Putinci *(Ranđelović et al., 2010)*.

Drought stress during flower induction or flowering has lower influence on yield reductions than drought stress during either pod formation or podfill *(Doss et al., 1974, Ranđelović, 2009)*. *De Souza et al. (1997)* concluded that long water stress from the beginning of growth stage R6 (early in seed filling) until maturity, shortened the seed-filling period (R7 occurred up to 7 days earlier) resulting in smaller seeds (32%) and lower yield (44%). Also, short periods of water stress during seed filling might decrease yield by up to 23% *(Brevedan and Egli 2003)*. Water stress during vegetative and reproductive stages of soybean growth did not

significantly affect the oil or protein content of seed and the fatty acid composition of the oil *(Dornbos and Mullen 1992)*. *Karam et al. (2005)* have found that the most critical stage for water stress is the R5 - R6 stages. *Dragović et al. (1993)* concluded that soybean is more sensitive to drought in R5-R8 reproductive stages than R1-R4 stages. Defensive mechanism of soybean against drought is also the fact that the root of soybean under drought conditions grows deeper into the soil and increases its density (*Purushothaman et al., 2013*). This increases water uptake and plant maintains a high potential for water and delays the dehydration.

Introduction of irrigation would enable the achievement of high and stable yields of soybean and the changing climatic conditions in Serbia. However, in Serbia is irrigated 1% of arable land *(Mandić et al., 2013)*. Therefore sowing soybean genotypes of certain maturity group should be adapted to agro-meteorological conditions of the site in order for them to express their maximum yield potential (*Ranđelović, 2009*). Soybean varieties of shorter growing period (0, I maturity group) are more suitable for planting in arid regions. Varieties of longer vegetation period (maturity group II) are recommended for growing in regions with favorable agro-meteorological conditions.

Conclusions

Harvested area, total production and grain yield of soybean in the Republic of Serbia for the 2009-2013 periods was the 160466 ha, 399350 tons and 2.48 t ha^{-1}, respectively. Harvested area, total production and grain yield of soybean in Serbia vary among years in range from 144386 ha (2009) to 170255 ha (2010), 280638 t (2012) to 540859 t (2010) and 1.73 t ha^{-1} (2012) to 3.18 t ha^{-1} (2010), respectively.

Production of soybean in Serbia is limited by drought in the summer months (from late June to early September) when are plants at flowering, pod-setting and grain-filling stages. Soybean is more sensitive to drought in R5-R8 reproductive stages (R5-R6 - seed development and R7-R8 - beginning and full maturity) than R1-R4 stages (R1-R2 - beginning and full bloom - and R3-R4 - beginning and full pod). Soybeans can tolerate short periods of heat and drought at early reproductive stages by aborting flowers and forming later. But the crop will not bloom indefinitely and under prolonged heat and drought.

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Uticaj vodnog stresa na produkciju soje

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Rezime

Soja je glavni izvor biljnih proteina i ulja u svetu. Soja se koristi u ishrani domaćih životinja i ljudi i u industrijsoj preradi. Soja je osnovna sirovina za izradu proteinske hrane za sve vrste i kategorije domaćih životinja. U svetu najveći deo proizvedene soje, oko 70%, koristi se za ishranu domaćih životinja.

U Srbiji površina pod sojom varira od 144386 do 170255 ha, a prinos zrna od 1,73 t ha^{-1} do 3,18 t ha^{-1}. Prinos zrna zavisi od količine padavina od kraja juna do početka septembra, kada su najveće potrebe za vodom (od početka cvetanja do kraja nalivanja zrna). Soja je najosetljivija na sušu tokom formiranja mahuna i nalivanja zrna.

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ORALLY PRESENTED PAPERS

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RESPIRATORY DISORDERS AND BIOSECURITY ON DAIRY FARMS

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Review paper

Abstract: Bovine respiratory diseases cause increased death losses, weight and production losses, with increase medication and labour costs. There is an interaction between the infectious agent(s), environment and immunity of the animal. Alone or in association with other pathogens, viruses are the important causes of respiratory diseases in calves: bovine respiratory syncytial virus, bovine herpes virus 1, parainfluenza virus 3, etc. Biosecurity plan for respiratory diseases have to include: (1) hazard identification: the specific infectious diseases that could pose a threat should be identified and listed in order of potential impact; (2) exposure assessment: the probable routes of exposure to identified diseases; (3) risk characterization: the level of exposure risk on the individual operation should be assessed for each disease and a prioritized list of the most important diseases to be targeted and the areas of greatest exposure risk; and (4) risk management: specific biosecurity and bio-containment protocols. “All-in all-out” practices or segregation of age groups might also limit agents’ transmission in multi-age facilities, as well as prevention of contact with other cattle, proper sanitization of equipment and surfaces between uses, wearing gloves and handling sick calves last. Addressing nonspecific factors related to respiratory health such as air quality, colostrum management and nutrition could also help limit the impact of respiratory pathogens, as well as appropriate vaccination.

Key words: biosecurity, respiratory disorders, dairy, farm

Introduction

Respiratory disorders, such as chronic or acute bronchitis and pneumonia of different aetiology, cause mortality and treatment costs, but most importantly, weight loss during illness and often protracted convalescence. Respiratory disease is a major animal welfare concern. Bovine respiratory disease (BRD) causes increased death losses as well as medication costs, labour, and lost production. Many different infectious agents may cause similar clinical signs (*Le Grand et al.* 2001).

Respiratory disease in cattle is an interaction between the infectious agent(s) (whether bacterial, viral or both), the environment, and immunity of the individual animal. The important viral and predisposing causes of respiratory disease are infectious bovine rhinotracheitis (bovine herpes virus 1) and bovine respiratory syncytial virus (BRSV); parainfluenza-3 virus is much less important. These viruses can cause disease by themselves or damage the defence mechanisms of the upper respiratory tract and predispose to secondary bacterial infections of the lungs (*Autio et al., 2007*). Livestock producers recognized animal movements as important routes for the disease dissemination (*Woolhouse and Donaldson, 2001*).

Biosecurity is essential aspect of food safety farm programmes. Planned and efficiently applied biosecurity measures lead to better health of dairy cattle of all categories and higher productivity, profit and environment protection (*Uhlenhoop, 2007*).

When a particular dairy farm is observed, infections by many ubiquitous agents have to be recognized as serious health problems and anticipated in farm biosecurity plan, such as parainfluenza or mycoplasmosis. If the presence of a particular agent is confirmed on the farm, risk assessment need to be performed (*Hristov et al., 2007; Stanković et al., 2014, 2015*).

Specific agents causing respiratory disorders

Alone or in association with other pathogens, viral infections are probably the main causes of respiratory diseases in calves (*van der Fels-Klerx et al. 2000; Smith, 2002*). Different viruses, such as bovine respiratory syncytial virus (BRSV), bovine herpes virus 1 (BHV1), bovine parainfluenza virus 3 (PIV-3), bovine coronavirus (BCoV), bovine adenoviruses or bovine viral diarrhoea virus (BVDV) are detected in most clinical cases that are investigated early after the onset of clinical signs (*Kapil and Basaraba, 1997*). They sometimes occur in combination and frequently also associated with bacterial invasion of the lung (e.g. with *Mannheimia haemolytica, Pasteurella multocida, Histophilus somni, Salmonella dublin* and *Arcanobacterium pyogenes*) (*Babiuk et al., 1988*). Bacteria and viruses

may also interact with *Mycoplasma* spp. to induce chronic or more severe disease in a synergistic manner (Thomas *et al.* 2002). Respiratory disease in cattle is often associated with the youngest animals such as calves, heifers or steers (Hartel *et al.*, 2004), mostly before the age of two years (*Crowe, 2001*), and respiratory disease is a major cause of losses, particularly in beef cattle production (*Prado et al., 2005*).

Economic loses

The economic impact of respiratory disease in adults seems of minor importance. Most cases of pneumonia are sporadic, and they are not a primary cause of culling at the herd level (Selman *et al*., 1977). Despite these facts, the practitioners are often facing cases of respiratory disease especially when dairy cows are outside on the pastures. These episodes raise many questions such as: the origin of the disease (viral, bacterial or parasitic), because of the contagious aspect at herd level, the persistent or chronic aspect of the cough, an aspect always unacceptable for the farmer, the impact of milk losses and of direct losses (cost of the treatment), the strategy of diagnosis and control (*Maillard et al., 2006*).

For instance, *Mycoplasma bovis*, an important contributor to major cattle diseases - mastitis and BRD, was estimated to be very expensive disease for the USA dairy industry (*Rosengarten and Citti, 1999*), through reduced production, drugs and labour for treatment, death and culling losses, diagnostic and control measures and non-pathogen-specific preventive measures. Because disease tends to be chronic, costs per case are typically high relative to other pathogens (*Fulton et al., 2009*). In addition to economic costs, there are important animal welfare consequences (*Hristov et al, 2009*), as often chronic and poorly responsive to treatment.

Disorders of the adult cows

Occasionally, as respiratory disorders of adult cows may occur tumours, caudal vena cava thrombosis, fibrosing alveolitis, fog fever (or Acute Bovine Pulmonary Edema and Emphysema), extrinsic allergic alveolitis (or hypersensitivity pneumonia - farmer's lung), all allergies, malignant catarrhal fever, ehrlichiosis, Q fever and general diseases of the old patient with a secondary respiratory impact, such as chronic cardiac failure or aneurysm of the carotid (*Chandler et al. 2001*).

Adult cattle can less easily be examined, outbreaks are often observed outside, and not in stables, clinical observations are often limited to inconstant hyperthermia, cough, polypnea and milk yield drop, "classical" ways to assess the

diagnosis often give poor results, antimicrobial therapy is not so easily applicable, because of its cost (related directly to the weight and the number of animals) and of the withdrawal period (*Maillard et al., 2006*).

Enzootic disorders are associated with contagious agents or management practices or both (*Callan and Gary, 2002*). Microbial agents are more likely than other agents to be responsible of collective illness. In this case, the whole herd must be suspected (or certain groups of animals) to be exposed at the same time to the same agent, as well as all (or most part of) adult animals, mainly because of naive or incomplete immunity or absence of effective vaccination (*Valarcher and Hägglund, 2006*).

Sporadic diseases are represented by all allergies, caudal venal thrombosis, fibrosing alveolitis, tumors and chronic suppurative pneumonia. In most case sporadic cases are therefore diseases of the sole adults (*Maillard et al., 2006*).

Main chronic disorders in adult cattle include chronic suppurative pneumonia/pleuritis (pasteurellosis or mycoplasmosis), fibrosing alveolitis, extrinsic allergic alveolitis, sometimes vena caval thrombosis (50% cases) and tumors (*Breeze, 1985*).

As usual for chronic disease, history is of great importance, including the previous treatments. As these problems involve very often a small number of animals if not a single one, prognosis seems more important than diagnosis. Decisions for culling or not are expected by the farmer in order to avoid long-term, cost-effective and frequently inefficient additional treatments (*Maillard et al., 2006*).

Main acute disorders include IBR, rarely BRSV, and allergies including milk allergy, vena cava thrombosis (the other 50% cases), parasitic bronchitis, malignant catarrhal fever, fog fever, Q fever and ehrlichiosis (*Peek, 2005*). Even more so than for chronic disorders, laboratory assays are necessary for the diagnosis; these cases are among the most difficult to solve, as acute disorders in adult cows are often group problems and as they can be of various origins, various samples and laboratory assays have to be performed (faeces, blood sera, blood cells, lung washing; PCR, serology, *etc*.). Sometimes the farmer can acquire a feeling of “persistent” or “chronic” disease, such as ehrlichiosis or Q fever, because of a slow evolution within the herd in multiple animals, and because some of them may relapse (*Joncour, 2004*).

Disorders of the calves

Similarly to adults, BRD and diarrhoea are the most common and economically important diseases in calves. BRD is the most common and severe disease in calf-rearing herds (*Autio et al., 2007*). Various microorganisms have

been shown to be involved in BRD together with predisposing factors. The most important viral agents are BRSV, PIV3, (BVDV), (BCoV), bovine adenovirus (BAV) and bovine herpes virus 1, the causative agent of IBR (*Thomas et al., 2002; Hägglund et al., 2006*).

Example from Finland which is free of IBR (*Nuotio et al., 2006*), where BVD is very rare (*Rikula et al., 2005*) and *Mycoplasma bovis* has not been detected since 1982, points out that monitoring of the causes of respiratory disease is essential in prevention, very successful and prophylactic use of antibiotics is rare. Sick animals are mostly treated individually, although a vaccine containing killed BRSV, PIV3 and *Mannheimia haemolytica* were introduced after study of *Autio et al*. (*2007*), as well as *A. pyogenes, P. multocida, M. dispar, Ureaplasma diversum, M. bovirhinis* and *Fusobacterium spp*. in more than 50% of herds, while *M. haemolytica*, *Histophilus somni* and *Bordetella bronchiseptica* were found in less than five herds in Finland. Laboratory confirmation is necessary before embarking upon a vaccination protocol in the face of infection, and to prevent future problems, as well as a choice of antibiotic (*Autio et al., 2007*). Secondary bacterial invasion of the damaged respiratory tract frequently occurs, which makes treatment difficult. In many situations selection of cattle for treatment based upon raised rectal temperature is the most cost-effective practice (*Maillard et al., 2006; Bojkovski et al., 2012*).

Management of the calf and cow at birth has profound effects on the risk of neonatal disease. Mostly, calves become infected with respiratory pathogens within the first days of life (*Maunsell et al, 2011*). Calves are raised in enclosed housing on continuous-flow, custom calf-raising operations and assembled from multiple herds and from sales channels where the risk of heavy exposure to infectious agents is high, with direct contact with one another, limited physical space per calf, and high risk of transmission by people and equipment. If housed, ventilation is often inadequate, resulting in a high relative humidity; faecal material is concentrated, with high moisture content and without full exposure to direct sunlight. Vermin such as flies and rodents are often present in high numbers, and nutrition is provided by assembled feedstuffs of varying quality and nutritional value rather than from dam's milk. (*Barrington et al., 2002*).

Biosecurity principles in respiratory diseases control

Control of infectious diseases relies on increasing host resistance to infection, removing reservoirs of infection, and preventing contacts that result in transmission (*Smith and Grotelueschen, 2004*). Biosecurity plans, either formal or informal, are part of the overall approach to control of infectious disease. Such plan need to be created according recognized threats and directed to elimination /

minimisation of these threats through designed reaction on possible incident (*Hristov and Stanković, 2009*).

Biosecurity plans should include: (1) hazard identification: the specific infectious diseases that could pose a threat are identified and listed in order of their potential impact; (2) exposure assessment: the probable routes by which animals would be exposed to each of the diseases are identified; (3) risk characterization: the level of exposure risk on the individual operation is assessed for each disease and a prioritized list of the most important diseases to be targeted and the areas of greatest exposure risk for those diseases is then produced; and (4) risk management: specific biosecurity and biocontainment protocols for the operation are designed, implemented, and monitored (*Wiggins et al., 2007; Hristov et al., 2013*).

The introduction of new pathogens, or the spread of pathogens already present in the herd to new groups of animals, can have a devastating effect on the individual dairy operation (*Thomas et al., 2002*). In addition, several infectious disease agents commonly found in dairy heifers are zoonotic and their control has public health implications (*USDA*, 2003). The prevention and control of infectious disease in replacement heifers is therefore an important component of any herd health plan (*Stanković et al., 2013*).

The "all in-all out" system enables efficient cleaning and disinfection procedures between rearing groups. On the other hand, mixing of calves from different farms exposes the animals to a heavy infection load, including microbes to which their dams do not have colostral antibodies. BRD is the most common and severe disease in calf-rearing herds (*Autio et al., 2007*). Homebred dairy cattle are typically vaccinated before the start of the breeding programme. Such vaccination protocols should be an integral part of most veterinary herd health programmes. Single vaccination affords life-long protection. Regarding IBR, treatment is based upon veterinary advice. It is essential that the veterinarian is contacted as soon as disease is suspected because the first cattle affected are the most severely affected and accurate diagnosis, treatment, and timely vaccination are essential to prevent further losses. The introduction of infectious agents into dairy farms is possible through direct transmission (purchased cattle, reintroduced resident cattle and contact with contiguous cattle) or indirect transmission (fomites, visitors, other species, and biological materials). In the absence of eradication programmes for many endemic infectious diseases, bioexclusion is the key management process for risk reduction, (*Mee et al., 2012*), particularly by general husbandry practices, especially the ventilation system which is often wholly inadequate and correctly-timed administration of vaccines. Reducing stocking density, wherever possible, would improve the disease situation on most units. Details of these measures must

be included in the veterinary herd health plan (*Stanković et al., 2013; Stanković and Hristov, 2014*).

According to *Gonzales et al*. (*1992*), the best way to prevent respiratory infections is to maintain a closed herd or screen and quarantine purchased animals. For dairy herds, it is recommended that the bulk tank culture history of the herd of origin be examined when purchasing heifers or adults. If this history is unavailable, the bulk tank can be sampled at least 3 times spaced 3–4 days apart. Where possible, calf health records should be examined to determine if *M. bovis*-associated diseases such as otitis media have been observed. When purchasing lactating cows, testing for specific antibodies in milk might be useful to identify infected cows (*Gonzales and Wilson, 2002*).

Generally, basic control principles of reducing exposure and maximizing host defences can be used in the control of respiratory infections in calves. Surveillance for respiratory agents in calf facilities includes monitoring of health records and diagnostic testing (*Maunsell et al., 2011*). Infection *via* infected milk can be eliminated by pasteurization or by feeding milk replacer at 65°C in 10 minutes or 70°C for 3 minute (*Butler et al., 2000; Stabel et al., 2004*). Exposure to infected colostrum could be reduced by pasteurization, by not pooling colostrum, and by not feeding colostrum from cows known to be infected with *M. bovis*. Exposure to airborne pathogens could be reduced by good ventilation and low-stocking density. “All-in all-out” practices or segregation of age groups might limit transmission of pathogens in multiage facilities, as well as prevention of fence-line contact with other cattle, proper sanitization of buckets, housing, and other equipment between uses, wearing gloves, handling sick calves last could reduce fomite-mediated transmission and sanitation, using chlorine-, chlorhexidine-, acid-, or iodine-based disinfectants. Addressing nonspecific factors related to respiratory health such as air quality, colostrum management, nutrition, appropriate vaccination and control programs for respiratory viruses, as controlling other pathogens could decrease the risk of *M. bovis* coinfections (*Maunsell et al., 2011; Stanković and Hristov, 2014*).

Nevertheless, there is permanent problem on dairy cattle farms is misunderstanding or even deliberate neglect of the importance of systematic application of biosecurity measures by employees and/or owners, in respect of isolating and layout of individual buildings, the introduction of newly acquired animals in the herd, as well as the technological way of doing repetitive tasks such as feeding, milking or use of medical materials. Procedures for sanitation facilities, resources and animals are often not respected, as well as managing data related to the envisaged and applied biosecurity measures (*Pritchard et al., 2003; Stanković and Hristov, 2009; Stanković et al., 2010; Stanković et al., 2011*).

Conclusion

According presented, it could be concluded that respiratory disease in cattle is an interaction between the infectious agent(s), the environment, and immunity of the individual animal. Alone or in association with other pathogens, viruses are the main causes of respiratory diseases in calves, such as bovine respiratory syncytial virus, bovine herpes virus 1, bovine parainfluenza virus 3, bovine coronavirus, bovine adenoviruses or bovine viral diarrhoea virus. They sometimes occur associated with bacterial invasion of the lung. Biosecurity plan for respiratory diseases have to include: (1) hazard identification: the specific infectious diseases that could pose a threat are identified and listed in order of potential impact; (2) exposure assessment: the probable routes of exposure to identified diseases; (3) risk characterization: the level of exposure risk on the individual operation is assessed for each disease and a prioritized list of the most important diseases to be targeted and the areas of greatest exposure risk; and (4) risk management: specific biosecurity and biocontainment protocols for the operation are designed, implemented, and monitored.

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Respiratorni poremećaji i biosigurnost na farmama mlečnih goveda

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Rezime

Respiratorna oboljenja goveda dovode do povećanja stope smrtnosti, gubitka telesne mase i pada proizvodnje, uz uz uvećanje troškova rada i lečenja. Ona su posledica interakcije infektivnog agensa (ili više njih), okruženja i imuniteta grla. Sami ili udruženi sa drugim uzročnicima, virusi su glavni izazivači respiratornih oboljenja podmlatka, pre svega bovini respiratorni sincicijalni virus, herpes virus 1 i parainfluenca virus 3.

Plan biosigurnosti protiv respiratornih bolesti treba da uključi: (1) identifikaciju rizika: prepoznavanje i navođenje redom prema značaju specifična infektivna oboljenja koja mogu biti pretnja; (2) procenu izloženosti: verovatne načine kako su grla izložena; (3) opis rizika: nivo izloženosti treba proceniti za svako navedeno oboljenje i područja najveće izloženosti; i (4) upravljanje rizikom: uključivanje specifičnih biosigurnosnih mera i protokola.

Praksa "sve unutra-sve napolje" i razdvajanje starosnih grupa mogu umanjiti mogućnost prodora uzročnika, uz sprečavanje kontakta sa drugim govedima, pravilnu sanitaciju opreme i površina između dve upotrebe, nošenje rukavica i rad sa bolesnim grlima na kraju. Kontrola nespecifičnih faktora: kvalitet vazduha, unošenje kolostruma i ishrana je takođe od značaja, kao i pravilna vakcinacija.

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STAPHYLOCOCCUS AUREUS – IMPACT ON UDDER HEALTH AND MILK SAFETY

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Review paper

Abstract: *Staphylococcus aureus* is known worldwide as a frequent cause of mastitis in dairy cattle. It usually cause catarrhal-purulent mastitis and galactophoritis, which can be acute or chronic, with different clinical and pathomorphological changes. The most severe outcome of udder infection with *Staphylococcus aureus* is gangrenous mastitis. Not only that *Staphylococcus aureus* can cause subclinical mastitis, without visible signs of infection, sometimes there is no increase in SCC (somatic cell count) either. Consequently, there is reduced milk yield, changes in milk composition as well as the production of biologically less valuable milk that is not suitable for further processing. Also, there is a risk of transmission of *Staphylococcus aureus* causing mastitis, between dairy cows, humans and other animals on the farm. Increased resistance to antimicrobial drugs, occurrence of resistance to methicillin, production of staphylococcal enterotoxins (SE), PVL (Panton-Valentin leukocidin) and other virulence factors, contribute to increased *Staphylococcus aureus* pathogenic capacity. Except the problem of antimicrobial drug selection for the therapy, there is the problem of secondary resistance which can be transferred from one bacterial cell to another, not only within the same bacterial species, but between related species. *Staphylococcus aureus* is also a major cause of food poisoning among humans. Heat-resistant staphylococcal enterotoxins, which can remain biologically active after cooking and other processes, represent the risk for public health. Identification of all cows infected with strains of *Staphylococcus aureus* and the implementation of biosecurity measures on dairy farms are crucial for preserving the udder health and obtaining milk quality and milk products, safe for human consumption.

Key words: dairy, mastitis, MRSA, PVL, enterotoxin, resistance

Introduction

Staphylococcus aureus has frequently been found in environment. It is intrinsically physically and chemically robust and tolerates pH ranges from 4.5 to 9.0 and NaCl concentrations up to 9%. Heat resistance is dependent upon the surrounding matrices. *Staphylococcus aureus* suspended in 0.9% NaCl is rapidly inactivated at 46°C, however, when protected by proteins (such as in milk or in pus) it can survive for more than 50 min at 60°C (*Anonym., 2009*).

Staphylococcus aureus can also be found in food animals, dairy cattle, sheep and goats, particularly if affected by subclinical mastitis (*Stewart, 2005*). *Staphylococcus aureus* is known worldwide as a frequent cause of mastitis in dairy cattle.

Staphylococcus aureus is also a major cause of food poisoning among humans, due to the production of heat-resistant enterotoxins, which when consumed cause vomiting and diarrhea (*Stewart, 2005; Anonym., 2015*). It can also cause severe cutaneous and respiratory infections among humans.

Major virulence factors in *Staphylococcus*, determining its pathogenic capacity, are: adherence, antiphagocytosis, exoenzymes, immune evasion, iron uptake, plasminogen activator, secretion system and toxins.

Staphylococcal mastitis

Mastitis is in general one of the most expensive health problems in the dairy industry (*Boboš et al., 2013-b*).

Udder infections caused by *Staphylococcus aureus* are present on many farms and can appear in form of subclinical and clinical mastitis. Consequently, there is reduced milk yield, changes in milk composition as well as the production of biologically less valuable milk that is not suitable for further processing (*Radinović, 2008; Radinović et al., 2008; Boboš et al., 2008; Boboš et al., 2013-b*). Also, there is a risk of infection transmission to healthy animals on the farm, and risk for milk safety and public health in general.

Staphylococcus aureus usually cause catarrhal-purulent mastitis and galactophoritis, which can be acute or chronic, with different clinical and pathomorphological changes.

The most severe outcome of udder infection with *Staphylococcus aureus* is gangrenous mastitis. In intensive dairy production, in newly imported cows, gangrenous mastitis is common. The disease occurs almost regularly in early puerperium at first calving. It appears rarely or never in older cows. The course of disease is rapid, and the outcome for the udder is always fatal, and very often for the cow too. The disease occurs most commonly in herds with newly imported

cows, in the process of acclimatization, probably because of the fact that these animals are under stress and they are less resistant to pathogens in the new environment. The treatments almost never give the desired results, and in such herds recommended measure is vaccination.

In 1980, *Bishop et al.* stresses the fact that *Staphylococcus aureus* is responsible for 33% of the clinical cases of mastitis.

Problem with *Staphylococcus aureus* persists on dairy farms today, much less as decades before. One of the reasons could be that udder infections caused by *Staphylococcus aureus* can also apear in a form of subclinical mastitis, when the only sign is increase in SCC.

Today, there are a lot of methods for SCC, some of them are direct, some indirect, some need more time and equipment, and some can be performed on farm. One of quick \indirect method which is often used on farms is California mastitis test (CMT). Results of CMT can be helpful in finding of infected udder quarters.

In 1988, on dairy farms in Vojvodina province, *Boboš* has recorded 52% milk samples positive on *Staphylococcus aureus*. In some researches, about twenty years later, on the same teritory, there was about 43% positive samples (*Radinović et al., 2008; Radinović et al., 2009*). Acording to results of a research from 2014, on dairy farms in Central Serbia, *Staphylococcus aureus* was found in 56% of CMT positive milk samples (*Pajić, 2014; Pajić et al., 2014-c*). *Vieira-da-Motta et al.* (*2001*) found *Staphylococcus aureus* in 35% of CMT positive milk samples. In a research of *Jánosi and Baltay* (*2004*) it was found in 32.5%.

Not only that *Staphylococcus aureus* can cause subclinical mastitis, without visible signs of infection, sometimes there is no increase in SCC either.

Infected udders, including the udder skin, are considered for the main reservoir of *Staphylococcus aureus* on dairy farms (*Boboš and Vidić, 2005*). Sometimes, *Staphylococcus aureus* is isolated also from cow's skin with no udder infection.

On dairy farms affected with staphylococcal mastitis, the same strains of staphylococci were found in the udder, as well as at the milker's hands and on vessels for milking.

Heckmann et al. (*1997*) showed that milking equipment has been most frequently the source of antibiotic-resistant strains of *Staphylococcus aureus*. Using scanning electron microscopy they have found source of bacteria to be skin debris particles (hairs, desquamated epithelium) at the surface of teat cup liner.

Transmission of *Staphylococcus aureus* strains between human and cattle has been indistinguishable proved by genotyping methods (*Lee, 2003; Juhasz-Kaszanyitzky et al., 2007; Hata et al., 2010*). Cattle infected with *Staphylococcus aureus* act as a reservoir for later transmission of infection to other animals and humans (*Spoor et al., 2013; Anonym., 2014-a*).

Therefore, the importance of hygienic measures on farms, especially measures related to milking, is well known. There are also procedures for control, reduction and elimination of *Staphylococcus aureus*, as well as for other patogens on dairy farms. Many authors around the world wrote about the importance of biosecurity and prophylactic measures in fighting mastitis on dairy farms (*Katić et al., 1990; Boboš et al., 1991; Boboš and Plavšić, 2005; Boboš et al., 2012-a; Boboš et al., 2012-b; Boboš et al., 2012-c; Radinović et al., 2012*).

Identification of all cows infected with strains of *Staphylococcus aureus* and the implementation of biosecurity measures on dairy farms is crucial for preserving the udder health and obtaining quality milk safe for human consumption.

Enterotoxin production

Enterotoxins are low-molecular weight proteins (26900 - 29600 Da). Up to now, more than twenty different staphylococcal enterotoxins have been described, all share superantigenic activity, whereas, only few of them (SEA to SEI, SER, SES, and SET) have been proved to be emetic (*Le Loir et al., 2003; Omoe et al., 2013; Hu and Nakane, 2014*).

Staphylococcal food poisoning is an intoxication that results from the food consumption containing sufficient amount of one or more preformed enterotoxin. Symptoms of staphylococcal food poisoning have a rapid onset (2-8h), and include nausea, violent vomiting, and abdominal cramping, with or without diarrhea. The disease is usually self-limiting and typically resolves within 24-48h after onset. Occasionally it can be severe enough to warrant hospitalization, particularly when infants, elderly or debilitative people are concerned.

Acording to summary reports of European food safety authority and European centre for disease prevention and control, staphylococcal toxins are responsible for significant percent of foodborne outbreaks caused by bacterial toxins (Table 1).

In 2013, 12 EU member states reported 386 foodborne outbreaks caused by staphylococcal toxins. This represents 7.4 % of all outbreaks, an increase compared with 2012 when 14 MS reported 346 outbreaks caused by staphylococcal toxins (*Anonym., 2015*).

Table 1. Foodborne outbreaks caused by staphylococcal toxins from milk and dairy products acording to summary reports of EFSA for years 2011, 2012 and 2013 (*Anonym., 2013; Anonym., 2014-b; Anonym., 2015;*)

Year	Number of reporting EU member states	Number of reporter outbreaks	% of all outbreaks	Food vehicles in strong evidence outbreaks			
				Cheese	Milk	Other dairy products	Total
2011	15	345	6.1%	8.6%	no data	5.7%	14.3%
2012	14	346	6.4%	20%	2,9%	no data	22.9%
2013	12	386	7.4%	6.4%	3.2%	2.3%	11.9%

Asao et al. (*2003*) confirmed that SEA from dairy products was the cause of extensive food poisoning outbreak in Japan, in 2002.

In a study of *Boboš*, carried out in 1988 in Serbia, 9.77% enterotoxin-producing isolates *Staphylococcus aureus* from milk samples was found. There were 64.29% SECs, 28.57% SEA and 7.14% SEB positive strains (*Boboš et al., 1988; Boboš et al., 1991*). After 25 years, enterotoxin-production was determined in 6.67% strains of *Staphylococcus aureus* isolated from cow's udder from 48 dairy farms in Serbia and it was proven that all of them produce the SECs only (*Boboš et al., 2013-a; Pajić, 2014; Pajić et al., 2014-b*).

Some other authors reported about much higher percent of SE positive isolates orginating from cow's milk. (*Advan et al., 2005; Gücükoğlu et al., 2012*)

It should be mentioned, that in France, bovine strains of *Staphylococcus aureus* are more often SED producers (*Ostyn et al., 2011*).

Due to stability of staphylococcal enterotoxins which can remain biologically active after cooking and other processes, the toxins can be found in food in the absence of viable organisms, since the latter may be killed during food processing by, for example, cooking or by reduction in pH as occurs during the manufacture of cheese (*Anonym., 2009*).

Even though some *Staphylococcus aureus* strains carry gene for the synthesis of SE, the expression of this gene depends on several factors. With the proper milk cooling, the risk to human health can be significantly reduced (*Boboš and Vidić, 2005*).

Panton-Valentine leukocidin

Panton-Valentine leukocidin (PVL) is an exotoxin, specific for *Staphylococcus aureus*. It plays a significant role in the occurrence of necrotic lesions in severe skin infections as well as in severe necrotizing pneumonia.

In research carried out on the territory of Central Serbia, the presence of genes for the synthesis of PVL was determined in 8.1% *Staphylococcus aureus* isolates originating from milk samples (*Pajić, 2014; Pajić et al., 2014-c*).

In a study from Western Algeria in the presence of genes for the synthesis of PVL was found in about 9% isolates of *Staphylococcus aureus* derived from the udders of cows with mastitis (*Benhamed and Kihal, 2013*).

Resistance to antimicrobials

Numerous reports indicate the increasing resistance of *Staphylococcus aureus* to antimicrobials. Problem of antimicrobial drug selection for the therapy of specific infection is not the only problem resulting from antimicrobial resistance.

The secondary resistance can be transferred from one bacterial cell to another. It is usually mediated by plasmids in processes of conjugation and transduction, not only within the same bacterial species, but between related species.

The use of antimicrobial drugs in dairy cattle is under permanent control. This could be one of the reasons why *Staphylococcus aureus* isolates originating from cow's udder are usually significantly less resistant than isolates originating from humans (*Pajić, 2014*). Also, in a research in Serbia it was concluded that *Staphylococcus aureus* isolates originating from the udders of cows with subclinical mastitis were resistant to fewer antimicrobials than isolates originating from the udders of cows with clinical mastitis. (*Boboš et al., 2014; Pajić, 2014; Pajić et al., 2014-a*)

Methicillin-resistant *Staphylococcus aureus*

Methicillin is semisynthetic penicillinase-resistant beta-lactam antimicrobial drug. It is no longer used in clinical practice, but the term "methicillin-resistant *Staphylococcus aureus*" (MRSA) is used for *Staphylococcus aureus* strains which are resistant to all penicillins.

MRSA is a particular problem in hospitals and nursing homes, especially by immunocompromised patients (*Ćirković et al., 2013; Ćirković et al., 2015*).

MRSA strains have no greater pathogenicity than MSSA - methicillin-sensitive *Staphylococcus aureus* strains, but there is a problem of choice of

antimicrobial drug for therapy of the infection they cause. Sometimes, there is no antimicrobial drug which could be used, or the choice is significantly narrowed (*Boboš et al., 2014*).

Livestock associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) is considered to be zoonotically important because of its capacity to colonize a wide range of hosts (*Paterson et al., 2012; Spoor et al., 2013; Joshi and Devkota, 2014*).

In a research in Serbia, a MRSA strain was isolated from a cow with clinical form of mastitis (*Pajić, 2014; Pajić et al., 2014-c*).

Transmission of *Staphylococcus aureus*

Transmission of *Staphylococcus aureus*, causing mastitis between dairy cows, humans and other farm animals was proven by genotyping methods. (*Lee, 2003; Juhasz-Kaszanyitzky et al., 2007; Anonym., 2009*; *Hata et al., 2010; Spoor et al., 2013; Anonym., 2014-a; Pajić, 2014*).

Based on determination of phylogenetic relatedness between 86 *Staphylococcus aureus* isolates originating from cow's udder and from human pharingeal swabs in a research conducted in Serbia, it was concluded that two *Staphylococcus aureus* isolates originating from humans can be considered to be the ancestors of all the other isolates in terms of gene mutation rate given at respective period, although there was no direct contact between those humans and cows (*Pajić, 2014*).

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Staphylococcus aureus – uticaj na zdravlje vimena i na bezbednost mleka

Marija J. Pajić, S. Boboš, M. Radinović, Branka Vidić

Rezime

Staphylococcus aureus je širom sveta poznat kao čest uzročnik mastitisa krava. Najčešće izaziva kataralno-gnojni mastitis i galaktoforitis, koji može biti akutni ili hronični, sa različitim kliničkim i patomorfološkim promenama. Najteži oblik mastitisa koji izaziva *Staphylococcus aureus* je gangrenozni mastitis. *Staphylococcus aureus* može izazvati i subklinički mastitis, bez vidljivih znakova infekcije, ponekad i bez povećanja broja somatskih ćelija u mleku. Posledice mogu biti smanjenje prinosa mleka, promene u sastavu mleka, kao i proizvodnja mleka koje nije pogodno za obradu. Takođe, postoji i rizik od prenosa sojeva *Staphylococcus aureus* između krava, ljudi i drugih životinja na farmi. Povećana rezistencija na antimikrobne lekove, pojava sojeva rezistentnih na meticilin, produkcija enterotoksina, PVL-a i drugih faktora virulencije doprinose povećanju patogenosti sojeva *Staphylococcus aureus*. Osim problema izbora antimikrobnog leka prilikom terapije, postoji i problem sekundarne rezistencije koja se prenosi sa jedne bakterijske ćelije na drugu, u okviru iste vrste ili različitih vrsta srodnih bakterija. *Staphylococcus aureus* je jedan od glavnih uzroka trovanja hranom kod ljudi. Termostabilni stafilokokni enterotoksini, koji ostaju biološki aktivni i posle kuvanja i drugih procesa prerade, predstavljaju rizik za javno zdravlje. Identifikacija krava inficiranih sojevima *Staphylococcus aureus* i sprovođenje biosigurnosnih mera na farmama su od ključnog značaja za očuvanje zdravlja vimena i dobijanje kvalitetnog mleka i mlečnih proizvoda, bezbednih za ljudsku ishranu.

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HEALTH DISODERS OF CALVES AND COWS IN TIE-STALLS HOUSING SYSTEM

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Review paper

Abstract: The intensive rearing of cattle includes a whole range of technological processes that should enable continuous production and optimal use of production capacity. In such circumstances, newborn calves have to adapt to different environmental factors, including diet. The technology of growing calves diet was initially based solely on feeding colostrum and then milk. After that, the food introduced other feed (hay, feed mixture), but after three months the milk completely excluded from the diet. In high-yielding dairy cows metabolic disorders such as ketosis, fatty liver syndrome, puerperal paresis, rumen acidosis and laminitis, have been caused by many factors, such as unbalanced nutrition, poor posture and breeding.

Keywords: calves, cows, disorders, health, tie-stalls housing system

Introduction

The intensive rearing of cattle includes a whole range of technological processes that should enable continuous production and optimal use of production capacity. In such circumstances, newborn calves have to adapt to different environmental factors, including diet. The technology of growing calves diet was initially based solely on feeding colostrum and then milk. After that, the food introduced other feed (hay, feed mixture), to a three-month milk completely excluded from the diet. Colostrum contains proteins, essential and not essential amino acids, fatty acids, lactose, vitamins, macro- and microelements. In addition, colostrum contains non-nutritive ingredients like immunoglobulins, peptides, hormones, growth factors, cytokines, steroid hormones, thyroid hormones,

nucleotides, polyamines and the enzymes (*Arthington et.al. 2000; Bojkovski et al. 2005 a, b, 2012*). At high-yielding dairy cows metabolic disorders such as ketosis, fatty liver syndrome, puerperal paresis, rumen acidosis and laminitis, have been caused by many factors, such as unbalanced diet and inadequate breeding. *(Basarić-Dinić, et al. 1996; 1997; Bojkovski et al., 2008; Petrujkić et al 2011; Stanković et al. 2009, 2011; Radojičić et al, 2008, 2014).*

The aim of this review article is to provide information on health status of calves and high-yielding dairy cows on farms with tie-stalls housing system.

The health status of calves

Neonatal calves are subject to cardio-respiratory and metabolic disorders that can be diagnosed in the early weeks of life. Practice has shown that calves individually develop the ability to consume colostrum, after milk. In these moments it includes the structure and function of their digestive organs. In the course of the colostrum period, colostrum changes in their composition. Colostrum has a role in the creation of passive immunity, but also has the ability to newly born calf supplies from the carbohydrates, lipids, proteins, micro elements, vitamins, and other biologically active compounds. The content of active substances, biochemically specific hormones, growth factors, cytokines, polyamines, nucleotides at neonatal calves ordered causes biological effects. Insulin-like growth factors (IGF), which shows a high value in colostrum can calves to stimulate the development of the digestive system in particular its function in the neonatal period. Establish the concentration of growth factors and hormones in the greatest secretion of colostrum before parturition *(Arthington et al. 2000, Blum, 1987).* If colostrum store first 24 hours after calving leads to an increase in NEFA, where the concentration of cholesterol, essential fatty acids, provitamin decreases *(Zanker et al., 2001).* During the first power concentration is greatest then after the first and second day of a decline in their concentrtation *(Blum et al., 1987).* Diseases of calves in intensive farming are multifactorial etiology. The most frequent and the most important health problems on our farms stand out disorders of the digestive and respiratory *(Bojkovski et al., 2007b, 2011,2014).* The occurrence of infectious diseases of different etiologies have great significance unfavorable hygienic and microclimate condition accommodation and inadequate technological procedures in raising calves. The intensive rearing of calves requires constant control the health status of calves and adequate implementation of prophylactic hygiene and animal husbandry measure. Improvement health condition positively affects the performance and the state of well-being calves. Welfare taken with regard to housing conditions usually endanger inadequate ventilation, air velocity and temperature of the air, as well as

exposure to live agens. In addition to data on housing conditions for a complete picture of the state of well-being are taken into account nutrition and specific control procedures, such as a power mode or refusal calves. Analysis of the potential risks for the benefit of a farmer indicates more possible causes already present health and other problems in calves as well as the problems that may occur in the future *(Relić and Bojkovski, 2010; Christ et al., 2012; Bojkovski et al., 2007, 2010, 2011 a, 2011b).*

The health status of cows

Ketosis is a metabolic disorder that occurs in high-yield dairy cows, most often in the third or fourth lactation, during the first weeks after calving and in early lactation. The occurrence of ketosis contributes more factors, but the main reason besides physiological low blood glucose in ruminants and insufficient energy intake at a meal, which is needed for the early stages of lactation. Glucose in the blood is the most important energy source for the cow. However, glucose can easily and rapidly fermented in the rumen. When there is not enough energy to mobilize fat reserves and accumulate ketone bodies in excess in amounts, which may cause disturbances in the body. In such cases, there are signs of ketosis: loss of appetite, weight loss, reduction in milk production, but in severe cases and nervous symptoms. Understanding the physiological and metabolic processes, as well as the peculiarities of metabolism in ruminants, which occur in pregnancy and lactation, it is particularly important for the science and practice of nutrition, given that food is just a medium through which in normal circumstances, the body supplies the necessary nutrients to settle increased needs during the aforementioned physiological conditions. Therefore, the task of feeding a high-yield dairy cows to ensure, above all, good health and condition of animals, lactation for a period of about 300 days, as well as the growing number of lactation during operation, the maximum amount of milk optimal chemical composition, and bringing healthy and vital during a year. To high-dairy cows could respond to these requirements, conditions for its keeping, care, health, nutrition and above all, must be maximally adapted to the needs of the organism (meal aligned to milk production) and pushed to the limits of the ideal, to the extent possible modern cattle breeding. It is known that those errors that occur in the last third of pregnancy, become clearly visible and marked during the early puerperium. With metabolic aspect therefore, ketosis is a disorder of metabolism of carbohydrate and fat, where in the blocked β-oxidation of fatty acids, and the process is stopped at the level of acetyl-CoA. A decisive crossroads of many roads in inetrmediary metabolism, even in flows resynthesis of glucose and fatty acid degradation, is oxaloacetate. Since the process of synthesis of glucose peak is activated, the largest amount of oxaloacetate is'

seized 'and' implemented " way of gluconeogenesis. This results in the accumulation of large amounts of acetyl-CoA, which cannot be completely oxidized, and there is a joining of two molecules of acetyl-CoA and ketone bodies synthesis of the first, acetoacetic acid, and from it, and β-hydroxybutyric acid acid and acetone. Synthesis of ketone bodies, within the physiological range, occurs in healthy animals in the intensity of 25 g / h. Most of the tissue is able to use ketone bodies as an energy source, and the degree of their decomposition is proportional to the concentration in the blood to levels of 3.44 mmol / L *(Stamatović and Šamanc, 1983; Djokovic et al., 2010).* Ketone bodies are in excess accumulates in the blood and then excreted in urine and milk, and partly through the lungs. Secretion of ketone bodies organism that is already in an energy deficit, in addition to losing a large amount of energy, given that the ketone bodies still tied around 75% of the energy value of fat (*Bojkovski and Borozan 2007c; Radijičić et al, 2008, 2014; Krsmanovic al. 2013*). Summarizing the foregoing considerations, it is necessary to focus the attention of the diet that emphasizes its importance in the prophylaxis and treatment of metabolic disorders. Be sure to take these conditions (subclinical ketosis) can be monitored and regular monitoring of parameters of metabolic profile, which included some analysis of the assessment of the functional status of liver cells (plasma glucose, AST, total bilirubin, and albumin) and a valid interpretation of the received analysis, it is possible to liver hepatoprotective protect and prevent ketosis *(Radojičić et al., 2002, 2007, 2008, 2014).* Meals for lactating dairy cows are based on the use of a large number of different nutrients and meals, in relation to the dominant type of nutrients, can be cumbersome and semi concentrated. When deciding which type of meal to determine the factors that should be kept in mind as before productivity of all the animals, but also the possibility of providing adequate nutrients, as well as its price. Bulky meals are usually much cheaper, or support the production of 10-12 liters of milk. In the case of higher milk yield, which is the tendency of keeping dairy cows, it is necessary to provide the cow material substrate from which will be made biosynthesis of milk, which means the application of the diet of the more expensive and higher quality concentrate feed *(Radojičić et al., 2008).* An important moment in the planning of feeding cows the harmonization of relations bulky and concentrated in the ration, especially in the dry period and early lactation. High yielding cows required usage of greater amounts of concentrated feed and it is recommended to avoid forages with high water content (silage with less than 25 % DM, cellulose feed).Too much voluminous of these nutrients, or a small amount of energy and nutrients per unit mass, makes cow unable to consume a sufficient amount to meet their basic needs *(Radojičić et al., 2008).* The quality hay (meadow, legumes etc.) may be offered at will. In addition, concentrated part of the meal, adapted to production status, should be divided into several parts in order to achieve a more even the arrival of

carbohydrates in the rumen and their interpretation under the influence of micro flora. This is especially important in order to avoid or significantly reduce the occurrence of rumen acidosis. This way of feeding avoid the emergence of " peak " glycemic, which is inevitable in the nutrition of high-yielding cows fed mainly concentrated fertilizers *(Radojičić et al., 2008).* A particular problem in planning meals represents the harmonization of relations of some kind of carbohydrate that the animal enters a meal. It is necessary to emphasize the importance of cellulose, but also starches as precursors of propionic acid, a key mediator of gluconeogenesis, while the share of easily digestible carbohydrates, although they represent " instant energy, " carries with it the constant risk of metabolic disorders. The meal made up of 32.2% of hay, silage 11.1%, 21.8% of root nutrient, and 34.9% concentrated feed ensures optimum milk yield and satisfactory state of health. Increasing the share of concentrated feed at 45% increased milk yield by 22% in the first third of lactation. In line with the traditional way of eating cows, recommend related energy originating from the bulky and concentrated feed, the level of 55:45, 75:25 and 94: 6 in the first, second and third period of lactation, which in the appropriate indigenous provides optimal conditions milk yield and milk quality *(Radojičić et al., 2008).* The parameter should be taken into account is the daily food consumption, which is especially a problem in terms of stress and drastic physiological changes during late pregnancy, parturition and early lactation. In general, meals should be tasty and attractive for a cow, preserved organoleptic properties, hygienic and free of harmful impurities.. The mechanism of control meal includes current control (the rumen pH content, the amount of volatile fatty acids and levels of certain hormones such as insulin, glucagon, gastrin) and long-term control of consumption (physiological state, the status of nitrogen, factors external environment, photoperiodical and seasonal differences, the level of production and the overall needs of the energy). There are also the body weight of cows, genetic markers, health, competitive higher priority (thermoregulation), as well as factors related to palatability and lure meal *(Radijičić et al., 2008).* In heavy conditions (unbalanced diet in prepartal period) comes to depositing excess nutrients in the body and so-called depots - fatty liver syndrome. Under conditions of energy deficit and the consequent rapid mobilization of fat from the depot, which is common in fat cows, and too quickly the accumulation of free fatty acids in the blood and their deposition in the liver. The high concentration of free fatty acid in blood conditional decrease appetite, and reducing the consumption of animal reacts, which then leads to an energy deficit when thin cows rapidly even with a possible lethal accumulation occurs outcome. Liver fatty acid in the form of lipid infiltration and degeneration of hepatocytes. With such an additional burden on the liver loses its functional activity which contributes to the appearance of ketosis, or aggravation of reparation and restitution processes in heatocytes, for

complete recovery is realized only when the liver rid of excess fat, which is a slow and long process *(Radojičić et al., 2008)* "Fatty liver syndrome" is therefore an example of the energy imbalance caused by excessive consumption of food / energy and increased deposition of fat in hepatocytes, but also in other tissues such as subcutaneous tissue, and often uses the term "fat cow syndrome". When cows during the last lactation period and dry period eceives high - energy meals leads to fat storage. This situation has an impact on the occurrence of severe calving of placental retention, or even the appearance of metritis. When the exhibit loss of appetite, resulting accelerated mobilization, fat from the body's reserves and, consequently, increased formation of ketone bodies. Common to all herds with this diet is to have fertility problems, as reflected by long service periods and reduced conception. In heard with long calving interval, usually at the time of calving cows have fattening condition too. Ketosis is then one of the secondary diseases that occur in such conditions, but which requires a particular therapeutic treatment (parenteral application of glucocorticosteroids and glucose) which further economically burden the production of milk. It is therefore necessary metabolic profile as a measure of the detection of subclinical ketosis, conducted at least twice a year, in late pregnancy and early lactation *(Jovanović et al., 1987; Djoković et al., 2007, 2014; Radojičić et al., 2008, 2014).* The objectives to be pursued in order to prevent fatty liver syndrome, the provision of adequate, not overly energy-rich meals, in order to prepare the rumen microorganisms, the meals that will be following in lactation, giving dry cows, 2-3 kg of these nutrients, two weeks before calving. After calving concentrate should be increased by 1kg / day, to achieve peak production. It is necessary to give 2-4 kg of hay daily, in order to reduce fluctuations of microorganisms, which can occur during the change in the meal, as a result of the high demand in the early period of lactation. It is desirable to maintain the interval between calving for a period of 12 to 13 months, or years / calf, separated by dry cows from dairy cows, with the aim to reduce the potential for "fatty liver syndrome" and to maintain a high lactation *(Radojičić et al., 2008, 2014; Djoković et al., 2014).* It is known that there may be the need of a number of inseminations and prolonging the cycle between calving in cows fed with a high content of easily soluble protein (17% protein with 75% solubility), which is also called syndrome of infertility. As a result of such a diet high contents of ammonia in the rumen, which is excreted through the urine or via milk. The increase in the content of ammonia nitrogen in milk, urine and body fluids, is a result of the need for an increased number of inseminations. Jon ammonia in excess operates toxic to the embryo and prevents the implantation and development of the fetus. Recommendations should relate to the reduction of accumulation of ammonia nitrogen, as a result of eating excessive amounts of protein. It is therefore necessary to avoid food with high amounts of protein, especially easy soluble

protein, and provide the necessary amount of energy (in conformity ratio of protein: energy), which is the main objective norms compliant food *(Petrujkić et al., 2011; Radojičić et al., 2008).* In addition to the importance of eating well-balanced meals are very important needs of micro-organisms in the rumen contents. The needs of microorganisms in the amino acids and energy, as well as the range of pH in the rumen (which is physiologically from 5.5 to 6.8) should be taken into account in determining the best meal at high-yielding dairy cows. Acidosis due to accumulation of lactic acid in an excess fermentation is a disorder that is manifested in several forms, depending on the amount of imbalance. Basically, rumen acidosis occurs when feeding highly soluble carbon-hydrated nutrients. When the hay are broken down in the rumen, under the influence of microorganisms, the fall in pH from 6.8 to a value of about 5 inhibits certain groups of microorganisms (protozoa and gram-negative bacteria) and facilitates the development of those bacteria that require an acidic environment. If the animal is long on such a diet or meal energy, acidosis can cause damage to the lining of the rumen, allowing bacteria formation. Complications arise as peritonitis, liver damage caused by switching of infectious agents in the blood stream, and consequently laminitis. Less important symptoms may arise as a simple refusal of food, which is subsequently adjusted to overeating, which may result in atony of the rumen, and the accumulation of excess fluid in the rumen. This together with an excess of fluid in the rumen results in signs such as dehydration, decreased appetite, reduction in milk production, reducing rumen contractions, sometimes dry dung, which may continue in the diarrhea. The aim should be to ensure a balance between the prevention of microorganisms. This can be achieved by feeding certain amount of food given to an equal intervals, avoiding sudden changes to the high-energy rations. Adequate amounts of concentrate and high quality roughage are necessary to achieve these objectives *(Bojkovski et al., 2007d; Radojičić et al., 2008; Radojičić et al, 2014.).* As a consequence of the transport of mineral substances (or inadequate intake or increased their loss) is the appearance of puerperal paresis or hypocalcemia or milk fever. Older cows have a reduced level of absorption of calcium, as well as reduced mobilization of calcium from the bones. In relation to the needs of the fetus for minerals and produced milk, these two facts lead to reduced opportunities to newly calved cows form physiological reserves of calcium in serum. In cows with puerperal palsy, decrease calcium levels (hypocalcemia) manifested with general weakness, "fall off my feet," sometimes coma and mortality, if the resulting disorder is not treated in a timely manner. In cows with puerperal palsy manifested and disorders in the form of indigestion and reduced absorption of nutrients. Factors that contribute to the occurrence of hypocalcemia are: reducing opportunities for older cows to quickly mobilize calcium from the bones or absorption from the small intestine, a decrease in

appetite that occurs after calving further deepens this problem (*Radojičić et al, 2008, 2014; Djokovic et al. 2014*). We must therefore allow just before calving physiological consumption, flow and absorption of nutrients. It is known to regulate the maintenance of physiological levels of calcium, calcium homeostasis is strictly regulated with several mechanisms (kidneys, skeletal system, with the participation of thyroid gland hormones and vitamin D).

Imbalance mineral matter immediately after calving and in older high-producing dairy cows, most often leads to so-called typical hypocalcemia or infrequently and so atypical parturient paresis in which the level of phosphorus domintan drop, and / or even less frequently recumbent cow syndrome.

Prophylaxis and recommendations in this regard related to the increased attention in the way of keeping and feeding in the dry period. Most metabolic diseases occurs at the end of pregnancy or in early lactation, and are caused by the troubled relations of energy and minerals in the diet. So have appeared recently and of the "syndrome of infertility" in feeding high protein diets in the dry period, and which affects negatively in the later stages of lactation or as a problem following conception (*Radojicic et al., 2008; Petrujkić et al. 2011*). Therefore up to three weeks before calving highly pregnant cows should gradually translate and get used to the ration of lactating cows, in order to rumen microorganisms "prepare and develop" the conditions for a new installment, as well as meal before drying off adding an equal relationship or even something more phosphorus than calcium, in order to timely launched moblisation calcium from reserves *(Basarič-Dinić et al., 1996; Basarič-Dinić et al.,1997).* It is also possible and prepares acidic or anionic meals. Such a meal reduces the incidence of milk fever and by a certain acidification that maximizes ionization of calcium in the intestines and improves its absorption and probably improves the ability to transport and the adoption of calcium from the bones. Another measure that can be taken is to add a meal of vitamin D. This is a good method if the time of calving knows exactly, but if these meals provide for a prolonged period can cause poisoning in vitamin D. Thus, the addition of vitamin D to frequent benefits, cannot be recommended, especially injectable preparation, because vitamin D has a structure and catabolism similar to steroid hormones, which can have a negative effect on the situation hepatocyte, who are otherwise significantly damaged in high-producing dairy cows fed unbalanced diets. An important moment in the planning of feeding cows the harmonization of relations bulky and concentrated work meals. Extremely high needs cows with high milk production require usage of greater amounts of concentrated feed, with a consequent reduction in crude fiber content in the diet. Because too voluminous such nutrients, or a small amount of energy and nutrients per unit weight, the animal is unable to consume a sufficient amount for the settlement. In this respect we should bear in mind that: a meal poor in calcium

weeks before calving intensifies metabolism of calcium, to provide meals with adequate amounts of calcium immediately after calving creates favorable conditions to maintain physiological levels of calcium, as well as the need to provide sufficient and / or increased amounts of magnesium, phosphorus and vitamin D. Meals with a low amount of calcium a few weeks before calving prevent puerperal paresis prepartal activation of bone system and the intestinal canal. It is recommended that two weeks before parturition benefits meal with less than 80 g Ca, P 60 g and about 35,000 IU of vitamin D. It is very important to solve the issue choices nutrient poor in calcium and assemble an adequate meal which settles other needs of the animals in the last weeks of the dry period. Hay and silage are in this case not suitable because of the abundance of calcium and nutrients as elections are used potatoes and by-products of potatoes, other root-tubers feed, brewery spent grain, meal of maize germ, bran and corn meal. Immediately after calving, when it is necessary to offer animals a meal rich in calcium, the account can come beet pulp, hay and silage (grass, legumes), as well as the addition of mineral nutrients as a source of calcium. Practical lack of such a diet is a necessity to ration after calving is changing gradually, as it is known that sudden changes in ruminants' meal can cause a disorder of micro-organisms and lead to indigestion. Compere meals applied in a series of trials aimed at testing the possibilities of preventing puerperal paresis, it was observed that increasing consumption in the dry period caused a greater incidence of the disorder. Cows with higher consumption prepartal demonstrated the labile calcium homeostasis and require additional treatment, while the animals that are fed small amounts of food, spontaneously recovered. Pursuant to the results, it is recommended that consumption in prepartal period increased by about 20% compared to the need to sustain life. Practical experience shows that on some farms there are cases of puerperal paresis without express hypocalcemia, or with severe hypomagnesaemia. Magnesium deficiency shows up as a significant factor in the formation of puerperal paresis, disrupting calcium homeostasis, because it reduces the level of calcium mobilization after calving, and considering that the mobilization of magnesium from the depot significantly slower than the calcium mobilization, are often the symptoms that resemble pasture tetany. The ratio of calcium and phosphorus in the diet significantly affect intestinal absorption, bone mobilization of calcium and the amount of available, but the recommended ratio of Ca: P of 1: 1. One of the options to prevent the appearance of puerperal paresis is changing the relationship of anions and cations in the meal. Increase acidogenic minerals compared to Alcalogenic a meal before calving has a positive effect on turnover of calcium (*Radojičić et al. 2008, 2014*). Laminitis represents aseptic inflammation of the hoof dermis. As the causes of the disease in addition to mechanical overload hoof statements are toxic and causes, and the formation of the allergic basis

(Bojkovski et al., 2013; Djokovic et al., 2014). Longer giving easily digestible concentrate feed, to acidosis in the rumen, sudden change of food, especially food green barley, oats, fresh trimmed young legumes and feeding moldy food, can lead to the development of laminitis. Laminitis is often the result of influence of a large number of factors, such as metabolic and gastrointestinal diseases, birth stress, mastitis, metritis, without docking or rugs with very little, without the possibility of movement, fattening, management, and poor nutrition. The meal, which leads to acidosis leads to laminitis, it is difficult to correct when the highest percentage of nutrients in a meal consisting of carbohydrate substances. It is believed that the vasoactive substances (histamine) that enter the bloodstream of the rumen to damage the hoof dermis. Metabolic disorder is caused by low rumen pH content and chained comes to the pathophysiology that ultimately result in ischemia dermis hooves and clinical symptoms of laminitis (disposal leg because of the pain, and sometimes forced lying) .Belive that besides histamine and bacterial endotoxins, lactic acid and other biologically active substances in the etiology of this disease. The amount of concentrated feed that the cow enters a meal, sometimes low rumen pH content and appearance of locomotor disorders, have a common etiopathogenetic background *(Dirksen, 1985; Radojičić et al. 2008, 2014; Bojkovski et al., 2011c, 2013).* There are claims that the presence of mycotoxins (fungi damaged food) can be involved in the etiology of laminitis. Aflatoxins each very present and most mycotoxins, leading to poor growth and poor reproduction, increased susceptibility to injury, reduced consumption and poor cellular and humoral responses of the immune system *(Dieckman and Green, 1992).* In a study in order to determine the relationship between aflatoxin contaminated feed, laminitis, and fertility observed lesions in the regions and hoof tarsus that led to lameness in 45 of a total of 300 high-yield dairy cows Holstein cows. In 27 cows are identified cystic ovaries, while the 10 cows had cystic ovaries with clinical equine cases. From this study came to the conclusion that aflatoxins enter the food (hay, alfalfa and concentrate) caused lameness (subclinical laminitis) and reduction fof fertility. Established amount of aflatoxin B1 (AFB1) in the hay was 4.1, 2.7 in alfalfa and concentrated feed 5.4 ppb *(Bilal, 2005).* Aflatoxin M1 (AFM1) is a metabolite of AFB1 contaminated feed and can be found in milk and milk products *(Patterson et al. 1980;Dieckmann and Green, 1992).* To make milk production and keeping dairy cows be profitable, it is necessary, with respect to all other prophylactic and zoo-technical measures, ensuring continuous animal feed quality, hygienic food meal balanced in all the necessary nutrients, in accordance with the accepted and scientifically verified recommendations

The pathogenic causes of disease in dairy cows

The presence of pathogenic microorganisms in calves and dairy cows was investigated on farms of high-yielding cows. We took rectal and nasal swabs. In microbiological laboratory was isolated the pathogenic microorganisms and in individual cases determined the adequate therapy. Also, we investigated the effect of different housing conditions and care for the presence of certain pathogenic microorganisms.

High-yielding cows during 305 days of lactation are constantly exposed to various factors that directly or indirectly affect the health of the animals. This situation imposes the need to constantly control the state of health, so that animals could survive in the production minimum five lactations. Today's technology of growing and keeping cattle in large agglomerations causes many health problems. In the calves were isolated bacteria's pathogens: *Escherichia coli, Pasteurella multocida, Mannhemia haemolitica* Truperella *pyogenes, Moraxella bovis, Streptcoccus spp, Cl. perfringens* Type *B (Bojkovski and Radojičič, 2004).* Diarrhea is a complex syndrome from the etiological point of view, developing as a result of environmental and nutritional factors and combination of diverse infectious agents. Based on examination of the rectal swabs, *E. coli* was isolated on a dairy breed cattle farm during the wintertime. According to the susceptibility test, appropriate antibiotic therapy was applied *(Ivanov et al., 1999; 2001).*

Moreover, respiratory diseases developing in the first several months of life are a major health problem. The airway diseases tend to develop as a result of inadequate hygienic conditions and complex synergistic viral and bacterial actions, expressed in the state of the impaired immune response of the organism. The most commonly reported viral causes *are BHV-1, PI-3, BRSV and BVDV*, while bacterial ones include *Pasteurella multocida, Mannhemia haemolytica* Truperella *pyogenes* Histophilus somni, and Mycoplasma bovis As for our tests performed on the nasal swabs of the calves affected with respiratory tract infections, the most common isolates were the following: *Pasteurella multocida, Mannheimia haemolytica,* Truperella *pyogenes, Haemophilus sp., as well as Klebsiella pneumoniae*, while in the lungs of the calves deceased due to bronchopneumonia, in addition to the above-mentioned species, mycoplasma was isolated as well *(Bojkovski et.al., 2014)* .

Presence of the infectious bovine kerato-conjunctivitis was evidenced in 4-month old calves, kept in the stalls. Ocular smears were obtained from the calves with clinically manifested keratoconjuctivitis and *Moraxella bovis* was isolated as the predominant bacterium, as well as *P. multocida, Streptococcus spp* and *Nocardia spp. (Džigurski et al., 2004; Bojkovski et al., 2007e).* In the advanced gestation phase, the ringworm was established as a zoonosis which represents considerable problem from the point of view of the human health. The ringworm is a dermatomycosis caused by a fungus *Trichophyton verrucosum* and it develops

mostly in calves and bullocks with lower physical fitness and impaired immune response. The causative organism inhabits skin surface causing, through its proteolytic and catalytic enzymes as well as endotoxin, parakeratosis and inflammatory changes. The factors predisposing onset of the disease include increased air humidity, overcrowding of the facilities and inadequate feed quality *(Bojkovski et.al. 2007f).*

Problems related to welfare and biosecurity in the cattle production

Given the numerous health problems listed calves and cattle, their welfare in intensive production is often called into question. Welfare of animals in terms of housing conditions often threaten inadequate ventilation, air velocity and air temperature, and exposure to pathogens and parasites *(Ostojić Andrić et al., 2011).* In addition to data on housing conditions, for a complete picture of the welfare state are taken to control the food and processes, such as for example power mode or rejection of calves. Analysis of potential risks to the welfare of a farmer indicates the possible causes for the present and other health problems in calves, as well as the problems that may arise in the future *(Relić and Bojkovski, 2011).*

Preventing disease in cattle also represent and protect their welfare. *Relić et al. (2006)* discuss the importance of measurements of the biological safety of dairy cows on farms, in order to prevent introduction of infectious material or its dissemination at the farm. Tests were conducted biosecurity conditions on the farms of high-producing dairy cows of different production technology and capacity, considering the position of farms in relation to possible sources bio-risk and distant environment. Test results indicated that in all tested farms there are serious shortcomings with regard to the possible introduction of infectious agents in the herd, and its possible spread to the environment. All studied farms were fenced, and the position of most farms was generally favorable. However, the problems were the lack of open space and green belt, uncontrolled presence of wild birds and rodents in facilities to keep cows and storing food, as well as contact employees with cows that do not belong to the farm which represents serious threat to health and livestock production farms *(Stanković et al., 2011).*

Conclusion

Diseases of calves in intensive farming are usually multifactorial etiology. The most important health problem in our farms high - producing dairy cows are separated diseases of the digestive and respiratory diseases. The occurrences of infectious diseases of various etiologies have great significance hygiene and microclimate, shelter and inadequate technological processes in education, particularly with regard to nutrition calves. The intensive rearing of calves requires

continuous monitoring of the health status of calves and adequate implementation of prophylactic, hygienic and animal husbandry measures. Improving the health status of a positive impact on the results of own production and the standing of welfare of calves. Profilactic measures to prevent disease in high producing dairy cows, should primarily provide physiological activity of microorganisms in rumen contents at the time when dry cows went into lactation. Request a balanced diet meal according to the production, separation of older cows from cows are in milk, regular control of metabolic profile as one of the ways of controlling health, and to dry cows permit the provision of sufficient quantities of high-quality roughage, at least two weeks before calving. After calving, the amount of concentrated feed should be increased gradually (about 1 kg per day) until the peak of milk production.

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Zdravstveno stanje teladi i krava u vezanom sistemu držanja

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Rezime

Intenzivan uzgoj goveda obuhvata čitav niz tehnoloških postupaka koji treba da omoguće kontinuiranu proizvodnju i optimalno korišćenje proizvodnih kapaciteta. U takvim uslovima novorođena telad moraju da se prilagode na različite činioce okoline, uključujući i način ishrane. U tehnologiji uzgoja teladi ishrana je u početku bazirana isključivo na ishrani kolostrumom, a potom mlekom. Nakon toga se u hranu uvode druga hraniva (seno, krmna smeša), da bi posle tri meseca mleko potpuno isključilo iz ishrane. Kod visoko - mlečnih kava metabolički poremećaji kao što su ketoza, sindrom masne jetre, puerperalna pareza, acidoza buraga, laminitis, su posledica uticaja mnogih činilaca, kao što su neizbalansirana ishrana, loši usovi držanja i nege.

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APPLYING SYSTEM APPROACH TO DEVELOP MIXED FARMING SYSTEM IN RECLAIMED DESERT LANDS IN EGYPT

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Abstract: The current study utilized system approach to characterize the existing mixed farming system in reclaimed desert lands, construct farm models that simulate the behavior of the current system with the objective of proposing different scenarios that can improve the overall efficiency of the system. Data were collected over one agricultural year from a random sample of 155 farms from three sites. Gross margin per animal unit, per feddan and B/C ratio were measured. The breakdown of livestock products showed that milk production revenues constituted 13.6%, 11.3% and 20.2% of the whole farm revenues, respectively. Results also showed that groundnuts crop considered the major source of income scored 38.2%, 30.4% and 29.9% of the whole farm revenues in the three studied sites, respectively. Applying the proposed scenarios the expected total gross margin, in comparison with the base run, in case of scenario 1, increased by 11.3%, 25.9% and 51.7% in the studied sites, respectively. While in case of scenario 2, gross margin increased by 6.7%, 56.4% and 54.9% and 2.4%, -2.1% and 9.1% in case of scenario 3, respectively.

Keywords: system approach, mixed farming system, gross margin.

Introduction

Egypt faces a serious challenge in maintaining food supply to exceed growing population. Therefore, efforts are focusing on improving agricultural production to bridge the gap between food supply and demands. One of the most important targets of the government policy is to reclaim desert lands located off the Nile Delta in order to increase the area of cultivated lands and find jobs opportunities for university graduates. Mixed farming system, including both

livestock and crop components, represents the dominant type of farming system in Egypt and is practiced in small scale farms. It is estimated that these farms contain about 95% of livestock population and produce about 70% of the total domestic milk production *(Abdel-Salam et al. 2008).* Interactions between those components often have major impact on the productivity and economical efficiency of the current farming system. In general, most farmers in the reclaimed lands use simple techniques in farming. The production of small scale mixed farms is still low and have to be raised to adequate standards to generate satisfactory income. This could be achieved through improving the efficiency of the whole system and providing farmers with suitable and effective technologies to improve the utilization of their limited production resources.

Considerable agreement exists among research workers about the general framework required for farming systems research *(Byerlee et al. 1980 and Nygaard, 1980)*. Most of authors considered a careful description of the existing farming system is an important first step to understand how the current system works. *Robert (1982)* stated that results of the characterization phase should provide sufficient understanding for development of hypotheses about production constraints. The present study followed system approach as described by *Morely (1982)* to; 1) characterize the current mixed farming system, 2) calculate farm budget to asses the economical efficiency of the system and 3) investigate the impact of proposed management scenarios on the overall efficiency of the current mixed farming system. Such a study would be useful in planning for comprehensive sustainable development of the mixed farming system in the studied reclaimed desert lands.

Materials and Method

The study areas

This study was carried out at Tahreer Province, a reclaimed desert land, located west of Nile Delta of Egypt, about 120 km North-West of Cairo. This area is classified as semi-arid conditions. Climate is characterized by hot dry summer followed by cool winter. Rainfall varies from season to season, with the major distribution of rainfall between November and March. This area contains a variety of small-scale mixed farming system of different farm size and cropping pattern. Three sites with respect to major farming schemes were identified to be the area of the study. Site 1, settlers are traditional farmers, site 2 included early employees and site 3, comprised university graduates.

Data collection

A random sample of 155 farms was investigated. A questionnaire was designed to cover available production resources, farming activities, annual costs and revenues. A field survey was performed and data on one agriculture year were collected. Data included the following variables: 1) production resources; farm size, family size and herd composition, 2) animal production performance; daily milk yield (DMY), lactation period (LP) and total milk yield (TMY), 3) crop production performance per feddan (feddan = 4200 m^2); main crop yield and by-product yield, and 4) Variable costs; which included hired labor, feeding, veterinary services, fertilizers, seeds and machineries in addition to whole farm revenues generated from livestock and crop activities.

Data analysis

Least squares analysis of variance technique were performed using fixed effects General Linear Model procedures of SAS (2001) to investigate the differences in production resources, livestock and crop performance among the studied sites and to derive estimates of technical coefficients for livestock and major crops production performance. The obtained estimates were used to construct the simulation models.

Farm budget

Farm budget is concern with organizing production resources to maximize profits, more often, family satisfaction. Preparation of farm budget allows planning for efficient use of resources and reflects the profitability of a farming system on an annual basis. Whole farm budget was adopted as a tool for financial evaluation of the current mixed farming system. The value of production is measured in terms of total revenues. This measure combines many different farm products into one measure. Revenues were calculated by multiplying the total quantities of the final marketable products by its current farm gate price. While the variable costs for different inputs were calculated by multiplying the quantities of inputs needed by its current market price. *Barnard and Nix (1993)* defined whole gross margin of an enterprise as its revenues less variable costs attributed to it.

Proposed scenarios

As the current study more concerned with livestock activities, three scenarios were proposed to enhance the overall efficiency of the current mixed

farming system namely; 1) Increasing total milk yield by replacing indigenous cows by crossbreds, 2) reducing livestock feeding costs by utilizing crops residues and 3) increasing milk revenues by producing high quality fresh milk or as simple home-made milk products.

Simulation Models

Simulation techniques were adopted to apply the proposed scenarios in each site. The sets of estimates produced by least squares analysis were used to construct deterministic simulated models to develop scenarios and analyzed by Simplex Linear Programming using Microcomputer Models for management and decision making *(Dennis and Dennis, 1988)* to re-allocate the available production resources to maximize the farm gross margin.

Economical efficiency criteria

To facilitate economical comparison among the three studied sites of different livestock and crops activities, return per unit of the limiting production resources was calculated by dividing the whole gross margin of the farm by the number of resource units needed. Changes in gross margin per feddan and per AU in addition to benefit/cost ratio (B/C) in response to the proposed scenarios were estimated.

Results and Discussion

Salient features

Characterization of the current mixed farming system in each of the three studied sites included; results of the field survey derived from the questionnaire, production resources, namely, farm and family size, herd composition, cropping pattern and current management practices. Results showed that mixed farming system is the dominating system and is practiced in small-scale farm size, where about 20% - 30% of the cultivated area is allocated to green fodder production. The remaining area is allocated to other cash crops. Most farmers own a small-scale herd of cows and/or buffaloes in addition to few numbers of small ruminants and poultry. Under the mixed farming system in the studied sites, three types of herds were differentiated according to the composition of herds: cow-herds, buffalo-herds and mixed-herds which included both cows and buffaloes. The cattle breeds raised in the studied area are native breeds and crossbreds. Results of the field survey for the distribution of these herds and major crops are shown in Table (1).

Table 1. Characterization of the three studied sites.

Herd composition:	Studied area		
	Site 1	Site 2	Site 3
Farm size (feddan)	4.6	13.8	15.4
Family size (person)	10.4	5.9	6.7
Herd size (AU)	2.32	2.40	2.84
Herd composition:			
Cow only herds (%)	24.7	20.0	27.4
Buffalo only herds (%)	27.4	35.0	43.5
Mixed herds (%)	47.9	45.0	29.1
Winter crop:			
Wheat (%)	42.7	31.3	20.6
Berseem (%)	36.1	19.3	16.5
Summer crops:			
Maize (%)	29.9	20.6	17.7
Groundnuts (%)	66.7	35.0	30.4

Current management practices

Animal production

Animals are kept in small enclosures connected to the family house. Animals were taken care of by family labour, mainly women. In most cases, cows and buffaloes served naturally with bulls available in the village. Mating was arranged in such a way that cows and buffaloes would calve within the clover season (October-May). Egyptian clover (*Trifolium Alexandrinum*) was the main source of feeding in winter. While in summer animals were fed on fodder maize, rice straw and crops by-product, in addition to some concentrates purchased from the market. Animals were hand milked twice, and the milk was used for family consumption either fresh or in the form of processed milk (cottage cheese, butter and ghee). Surplus fresh milk and/or milk products were sold at the village market or to the middlemen.

Crop production

The cropping pattern resulted from the interaction of farmers' objectives, natural factors, government policy, managerial capabilities and financial capacities. Allocation of land for various crops was left to the farmer decision. The sprinkling and soaking techniques of irrigation are the common irrigation systems. Nile water in the main source of irrigation water. Most of farmers used machinery,

particularly, in preparing the soil for cultivation. These equipments were owned by farmers or rented. Most of farmers in the current studied areas utilized chemical fertilizers, in addition to manure produced from their own farms. Results showed that irrigation water is mainly obtained from River Nile.

Technical coefficients

Animal production

Least squares means and standard errors of the technical coefficients (TMY, LP and DMY) for different breeds in the three studied sites are presented in Table (2). The obtained result showed that buffaloes in site 2 produced the highest TMY compared to those in sites 1 and 3, those differences accounted for 47.9% of site 1 and 44.2% of site 3. While native cows raised in site 3 produced more TMY compared to those in sites 1 and 2. In the same context, crossbred cows raised in site 3 produced more TMY compared to those of sites 1 and 3. These differences in TMY among different breeds may be due to different management practices and feeding regime in each site.

Table 2. Technical coefficients of milk production performance of the studied sites.

Trait	Reclaimed desert lands		
	Site 1	Site 2	Site 3
Total milk yield: (kg)			
Buffalo	957.8 ± 92.20	1417.1 ± 137.11	982.7 ± 72.58
Native cattle	599.1 ± 102.62	781.6 ± 240.06	862.9 ± 126.88
Crossbred	1110.4 ± 166.63	1227.5 ± 295.66	1414.6 ± 86.38
Lactation period: (day)			
Buffalo	179.1 ± 8.66	229.0 ± 12.87	207.9 ± 6.81
Native cattle	159.2 ± 9.63	154.7 ± 22.54	196.3 ± 11.91
Crossbred	188.1 ± 15.64	217.4 ± 27.76	202.1 ± 8.11
Daily milk yield: (kg)			
Buffalo	5.3 ± 0.36	6.2 ± 0.54	4.8 ± 0.29
Native cattle	3.8 ± 0.40	4.9 ± 0.95	4.2 ± 0.50
Crossbred	6.1 ± 0.66	5.3 ± 1.17	6.9 ± 0.34

Crops

The crop yiel/feddan is a composite trait resulting from natural factors, rotation design, weed control, soil fertility and structure, seedling rate, system of irrigation, cultivating date and timing of each operation. The least squares means and standard errors for yield per feddan of the major field crops in the three studied sites are presented in Table (3). The crops yield per feddan were 9.7 ardan, 5.5 and

4.9 for wheat, 8 ardab, 4.6 and 4.11 for maize and 24 tons for berseem for the three studied sites, respectively.

Table 3. Crop production per feddan in the three studied area.

Crop	Reclaimed desert lands		
	Location 1	**Location 2**	**Location 3**
Winter crop:			
Wheat (Ardab)	9.7 ± 0.30	5.5 ± 0.75	4.9 ± 0.36
Berseem (Ton)	24.0	24.0	24.0
Summer crop:			
Maize (Ardab)	8.0 ± 0.31	4.60 ± 1.11	4.11 ± 0.57
Groundnuts (Ardab)	11.5 ± 0.44	5.2 ± 0.84	8.3 ± 0.51

Farm budget

The whole farm budget breakdowns of the three studied sites are illustrated in figures (1), (2) and (3). The results revealed that 26.6%, 23.4% and 20.8% of the total variable costs are going to the animal production activities, while 73.4%, 76.6% and 79.2% are going to the crop activities, respectively. Out of animal production variable costs, feeding costs represented about 24.1%, 22.2% and 18.1%, respectively. On the other hand, livestock production revenues were 24.6%, 28% and 38.2%, respectively. The breakdown of livestock products showed that milk production revenues constituted 13.6%, 11.3% and 20.2% of the whole farm revenues, respectively. These variations may due to the different objectives of each farmer and herd size and composition. The breakdown of crops products, results also showed that groundnuts crop considered the major source of income scored 38.2%, 30.4% and 29.9% of the whole farm revenues in the three studied sites, respectively.

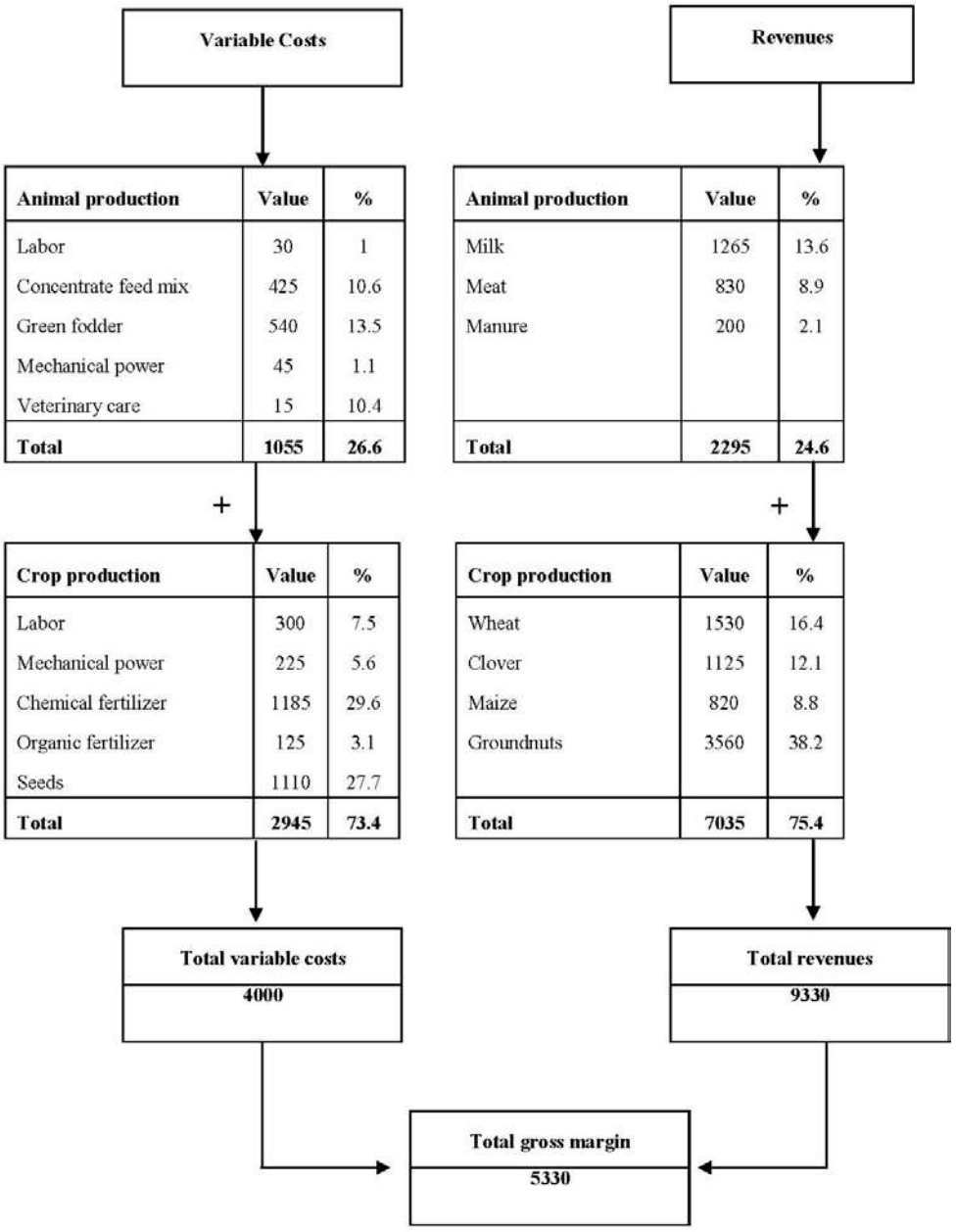


Figure 1. Breakdown of annual variable costs and revenues of crop / livestock farming system for location 1

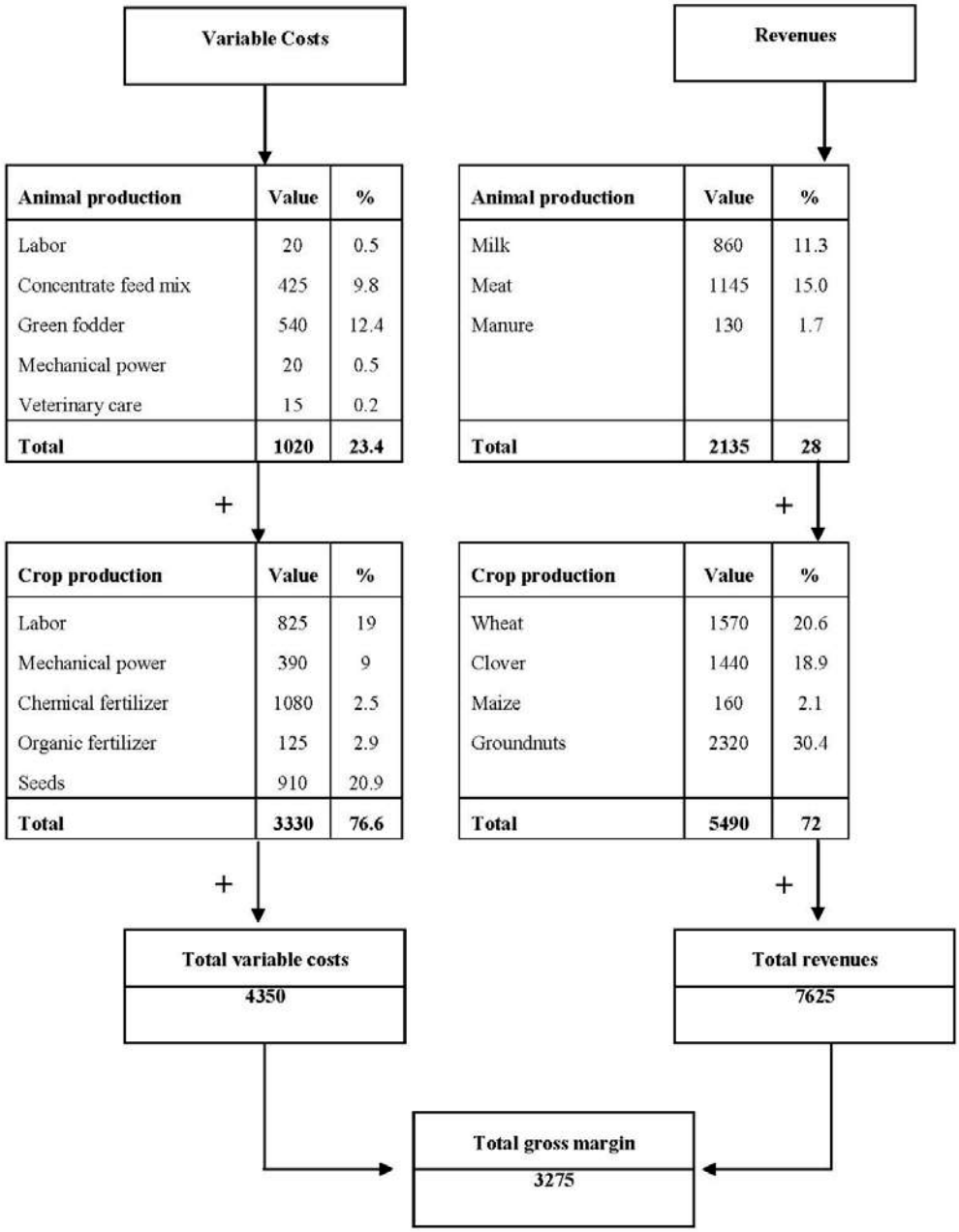


Figure 2. Breakdown of annual variable costs and revenues of crop / livestock farming system for location 2

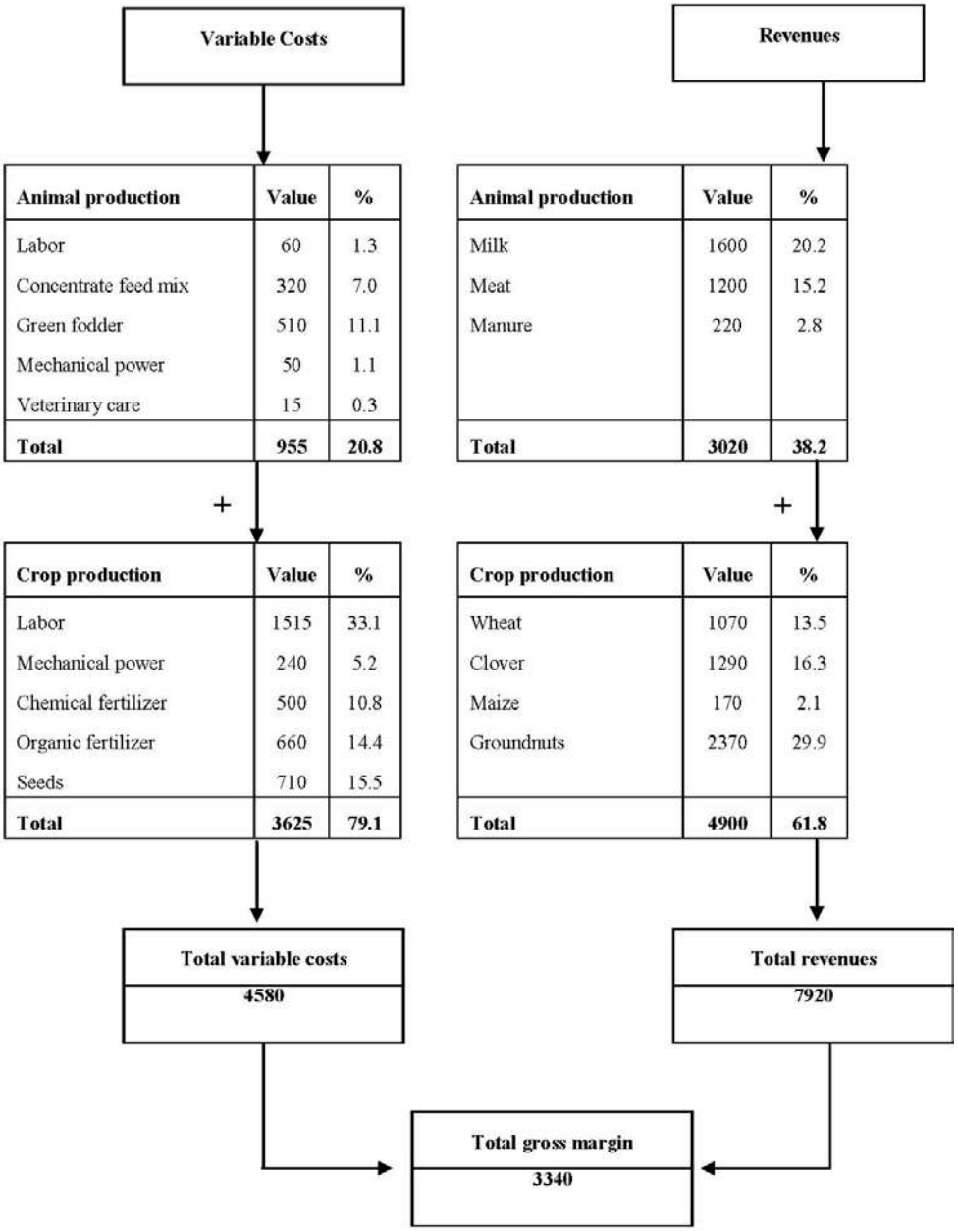


Figure 3. Breakdown of annual variable costs and revenues of crop / livestock farming system for location 3

Economical efficiency

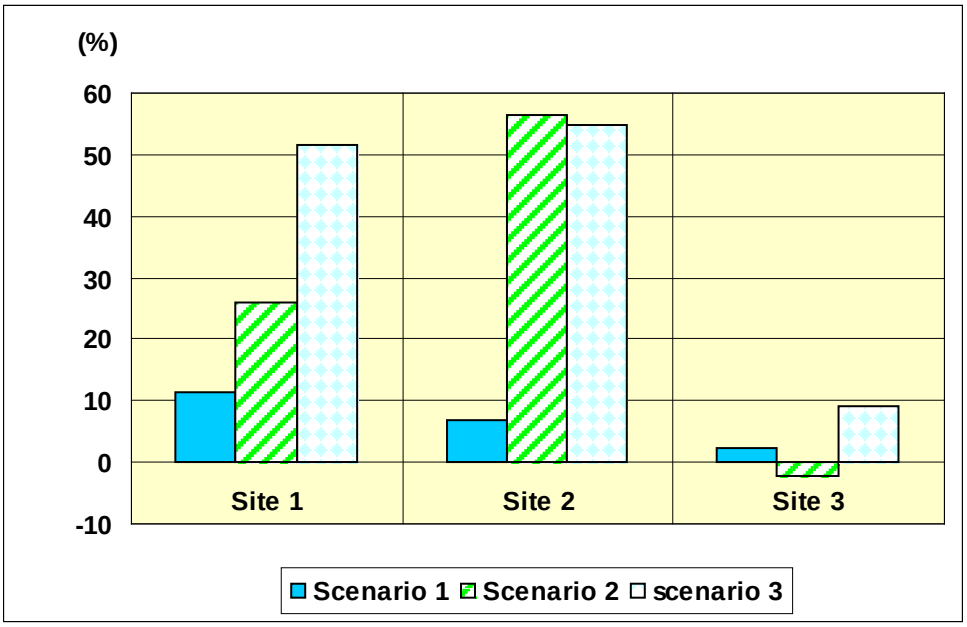
Indicators of the economical efficiency of the three studied sites are presented in Table (4). The obtained values of the economical efficiency revealed that site 1 is more efficient in utilizing farm resources than the other two sites this may due to proper management and appropriate allocation of the available resources.

Table 4. Economical efficiency of the mixed farming system in the studied sites.

Criteria	Site 1	Site 2	Site 3
Gross margin / feddan	1159	237	217
Gross margin / AU	2297	1365	1176
Overall benefit / cost ratio	2.33	1.75	1.73

Proposed scenarios solution

Results of the present study revealed that modeling with the aid of linear programming is a useful tool for farmers and decision makers. It is of great interest to notice that the response of proposed scenarios didn't have the same impact on the total gross margin of the farm and economical efficiency. The different responses could be attributed to the variations of the major characteristics, production resources, cropping pattern, management practices and constrains existed in each site. Applying the proposed scenarios the expected total gross margin, in comparison with the base run, in case of scenario 1, increased by 11.3%, 25.9% and 51.7% in the studied sites, respectively. While in case of scenario 2, gross margin increased by 6.7%, 56.4% and 54.9% and 2.4%, -2.1% and 9.1% in case off scenario 3, respectively (figure 4).



Primena sistemskog pristupa u razvoju kombinovanog poljoprivrednog sistema u pustinjskim regionima u Egiptu

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Rezime

Trenutna studija koristi sistemski pristup u karakterisanju postojećeg mešovitog poljoprivrednog sistema u regionima gde su pustinje pretvorene u korisno zemljište , izgradnju modela farmi koji simuliraju ponašanje trenutnog sistema sa ciljem predlaganja različitih scenarija koji mogu da poboljšaju ukupnu efikasnost sistema. Podaci su prikupljeni tokom jedne poljoprivredne godine od slučajnog uzorka od 155 farmi sa tri lokaliteta. Merene su bruto marža po uslovnom grlu, po fedanu i B/C odnos. Analiza stočarskih proizvoda je pokazala da prihodi od proizvodnje mleka predstavljaju 13,6%, 11,3% i 20,2% od celog prihoda farmi, respektivno. Rezultati su takođe pokazali da je usev kikirikija, koji se smatra glavnim izvorom prihoda, postigao 38,2%, 30,4% i 29,9% od ukupnog prihoda farmi u tri proučavna lokaliteta, respektivno. Primenom predloženih

scenarija, očekivana ukupna bruto marža, u odnosu na bazu, u slučaju scenarija 1, povećana je za 11,3%, 25,9% i 51,7% na ispitivanim lokacijama, respektivno. Dok je u slučaju scenarija 2, bruto marža povećana za 6,7%, 56,4% i 54,9% i 2,4%, -2,1% i 9,1% u slučaju scenarijao 3, respektivno.

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THE BLOCK OF ZYGOTE DEVELOPMENT IS RELATED WITH CULTURE CONDITION

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Abstract: Despite progresses in in-vitro embryo production, the embryo block have still occurred in plenty of zygotes. The lack of understanding of developmental block mechanism and its relation with suboptimal IVEP conditions encouraged us to investigate the effect of somatic cell co-culturing with different culture media on this feature. The cows ovaries collected from local abattoir was transferred to laboratory to collect cumulus oocyte complexes (COCs). The selected COCs were matured in five media (TCM199, ham's F12, mSOF, follicular fluid and chick embryo amniotic fluid) at 3 somatic cells monolayers (cumulus, granulose and oviduct epithelial cells) and without somatic cells in factorial design for 24 h under mineral oil. After IVF, the presumptive zygotes were cultured in own treatment which matured. The cleavage and block rate was recorded at days 2-3 and 8 PI, respectively. The culturing medium containing ChEAF (chick embryonic amnion fluid) resulted in most cleavage rate (49.50±11.51) and least blocked zygotes (51.00±13.04) than other media ($p<0.001$) and cow's oviductal epithelial cells (COECs) from the somatic cells factors was shown most cleavage rate (45.31±12.96) and least blocked zygotes (50.85±14.24) from other somatic cells ($p<0.001$). Zygotes which co-culture by COECs in ChEAF medium were significantly ($p<0.001$) different from other treatments in respect of cleavage rate (57.54±10.36) and decreasing of zygotic development block (41.31±5.98). In conclusion, the development of embryos are effected by in vitro culturing conditions and in this study ChEAF with cow oviductal epithelial cell resulted in low developmental block.

Key word: cleavage, co-culture, developmental block, media, zygote

Introduction

In vitro embryo survival decreases from cleavage to blastocyst which known as embryonic developmental block, occurs in most mammalian's embryos. Cattle, sheep and goat embryos block at 8-16 cell stage (*Meirelles et al., 2004;*

Tervit et al., 1972; Sakkas et al., 1989) while porcine and rat embryos cease development at 4 cell stage, and murine embryos block at 2 cell (*Schini and Bavister, 1988*). Despite progresses in in vitro embryo production (IVEP), the embryonic block have still occurred in plenty of zygotes. Actions of some factors which involved in oocyte maturation, fertilization, zygote and embryonic development have been identified (*Li et al.*, 2000), but majority of them have remained far from complete identification. Therefore, in vivo physiological conditions could not completely reflected by today's in vitro embryo culture systems (*Buratini Jr and Price, 2011*) which resulted to low blastocyst formation rate (*Rizos et al., 2002*) and block of zygotic development.

Co-culturing of oocyte and zygotes with somatic cells might be a rational approach to overcome this problems. However, it has been demonstrated that type of somatic cell and culture media significantly effects on co-culture efficiency (*Gandolfi et al., 1986; Rexroad et al., 1988; Duszewska et al., 2000).* Different culture media have a different potential to cell and zygotic growth and development. *1988*) showed that TCM-199 supplemented with 10% FCS was better than Ham's-F10 supplemented with 10% FCS for co-culturing ewe one and two cell zygotes with oviductal epithelial cells (OECs) in respect of blastomere cell numbers at day 5 of co-culturing. However, in co-culturing system the fundamental problem is culturing system which need to support not only somatic cell deviation and growth but also embryonic development (*Leppens et al., 1996*).

Therefore, the lack of understanding of developmental block mechanism (*Rief et al., 2002*) and its relation with suboptimal IVEP conditions encouraged us to use somatic cell co-culture with different media to find best medium with regard to investigated somatic cells to improve zygotic development and subsequently reduce embryonic developmental block.

Material and Methods

The collected cow's ovaries from local slaughterhouse were transported to the laboratory within 2-3 h in CR1 medium at 4 ^{o}C. The COCs were rescued by slicing method (*Bohlooli et al., 2015*) and only oocytes with at least 3 layers of compact cumulus cells selected to maturation. The selected COCs was co-cultured with and without somatic cells (cumulus, granulosa, cow oviductal epithelial cells) monolayer in different experimental media (TCM-199, Ham's-F12, mSOF, TCM+50% FF and TCM+50% ChEAF; all media were supplemented with 10% FCS and antibiotics) at 38.5 ^{o}C in a humidified atmosphere of 5% CO_2, 5% O_2 and 90% N_2 for 24 h. The matured oocytes were co-cultured with sperm ($1x10^6$/mL)

for 18 h in SOF-fert media and then, the presumptive zygotes were transported to medium and somatic cell monolayer which matured at it, to subsequence development.

The cleavage and blastocyst formation rate was recorded at day 2-3 and 8 PI (post insemination), respectively. The difference between number of cleaved zygote and number of blastocysts was calculated as blocked zygotes. The percent of blocked zygote was calculated from following equation:

$$\%\ of\ blocked\ zygote = \frac{number\ of\ blocked\ zygote}{number\ of\ cleaved\ zygote} x100$$

To collect oviduct epithelial cells, three – four cow oviducts were collected from slaughtered animals and cleaned from surrounding tissue. The mucosa was mechanically expelled from oviducts by a sterile glass slide. After three times washing in Hepes-TCM-199 supplemented with 10 % fetal calf serum and antibiotic, epithelial oviduct cells were layered on 60 % percoll and centrifuged at 500×g for 15 minutes to separate the clumps of epithelial cells, erythrocytes, and leukocytes. The cells were seeded in micro droplet under mineral oil and incubated in environmental condition as mentioned to maturation. The granulosa cells were collected from supernatant of ovary slicing medium after 5 minute of sedimentation time. The granulosa cells dispersion was fulfilled by 0.2 % hyaluronidase in 20-30 minute and after centrifugation the cell pellet was layered on 60 % percoll to separate granulosa cells from cell clump and microbial contaminations. After final washing, the granulosa cells were seeded and incubated as oviduct epithelial cells. The oocytes surrounded by cumulus cell were transferred to 0.2 % hyaluronidase solution and vortexed after 10 minute to collect cumulus cells. The oocytes were discarded and cumulus cells were seeded after cleaning by percoll as mentioned above.

The study was performed by 4x5 factorial experimental designs which in somatic cell factor had 4 levels and medium factor with 5 levels. The test of normality was performed by Shapiro-Wilk w-test. Following Arcsine square root transformation, analysis of variance was performed as well (ANOVA) in order to assess significance of differences. The Duncan Range Test was used for comparison of means ($p<0.05$). The statistical analysis was performed by R program and Duncan test by using Agricolae package program (*Seefeld and Linder, 2007; de Mendiburu, 2014*).

Results and Discussion

The cleavage rate of oocytes was significantly affected by co-culturing of different somatic cells in different media ($p<0.001$). As shown in table 1, the oocytes which co-cultured with COEC in ChEAF medium (57.54±10.36) expressed highest cleavage rate, while this rate was lowest in mSOF medium without any somatic cells (21.65±7.58). When factors were considered independently, the medium ($p<0.01$) and somatic cell ($p<0.01$) factors had a significant effects on cleavage rate. The figure 1 has shown that highest and lowest rates were obtained from ChEAF (49.50±11.51) and mSOF (27.99±9.85) media, respectively. Regardless of medium effect, the co-culturing of COCs with COEC resulted in higher (45.31±12.96) cleavage rate whereas lowest cleavage rate in granulosa cell (38.00±9.90) co-culture media (Figure 2). There was no significant interactions between factors ($p=0.5154$).

Table 1. Effect of different media and somatic cell co-culturing on cleavage and zygotic development block rate

Medium	Somatic cells	Cleavage % ± SD	Blocked % ± SD
TCM199	*Cumulus*	49.30^{abc}±8.80	68.69^{ab}±10.66
	Granulose	41.25^{bcde}±6.29	63.53^{abc}±4.49
	COEC	49.13^{abc}±9.00	47.65^{cd}±4.86
	Without SC	40.76^{bcde}±5.77	73.67^{a}±4.52
Ham's F-12	*Cumulus*	42.60^{abcde}±3.55	60.35^{abcd}±13.27
	Granulose	40.33^{bcde}±5.39	61.07^{abcd}±6.1
	COEC	50.41^{ab}±4.44	55.89^{abcd}±6.97
	Without SC	46.66^{abc}±7.66	72.98^{a}±14.65
mSOF	*Cumulus*	22.34^{f}±8.90	72.23^{a}±16.21
	Granulose	29.50^{def}±6.25	73.19^{a}±9.03
	COEC	38.45^{bcde}±8.00	60.35^{abcd}±21.96
	Without SC	21.65^{f}±7.58	75.15^{a}±8.91
FF	*Cumulus*	28.32^{ef}±5.23	56.66^{abcd}±8.16
	Granulose	34.18^{cdef}±10.54	60.41^{abcd}±7.21
	COEC	31.01^{def}±14.22	49.05^{bcd}±19.98
	Without SC	34.64^{cdef}±15.11	60.23^{abcd}±10.07
ChEAF	*Cumulus*	48.94^{abc}±14.04	55.8^{abcd}±4.82
	Granulose	44.72^{abcd}±14.29	47.91^{cd}±21.91
	COEC	57.54^{a}±10.36	41.31^{d}±5.98
	Without SC	46.78^{abc}±5.18	58.96^{abcd}±7.59

The developmental block rate also affected by treatment ($p<0.01$). As shown in table 1, the mSOF medium without any somatic cell had higher block rate (75.15±8.91), while the culturing of COCs in ChEAF with COEC resulted in lower blocked zygote rate (41.31±5.98). In term of factor's effect, the block rate was

affected by media ($p<0.001$) and somatic cell ($p<0.001$) factors. In term of medium factor, the mSOF medium resulted in highest rate (70.23±14.73) of development block while the lowest was observed at the COEC medium (51.00±13.04).

This study revealed that cleavage rate was superior and occurrence of early embryonic developmental block was lower at oocytes which co-cultured in ChEAF medium with COEC compared to other treatments which were tested in this study. This embryonic developmental promoting effect of co-culturing with COEC in ChEAF may be attributed not only to embryotropic effects of COEC and ChEAF, but also to ChEAF which may increase COECs proliferation in term and cleavage rate whereas decrease developmental block rate.

In term of the factors, the TCM199, Ham's F12 and ChEAF from medium factor resulted in high cleavage rate, but lower block in ChEAF. The lower cleavage rates were observed in FF and mSOF media as reported by *Romero-Arredondo's* (*1996*) study which in medium without FF resulted in higher cleavage rate (85±5.1) than those with 20% (36±12.2) and 40 % of FF (45±18.9).

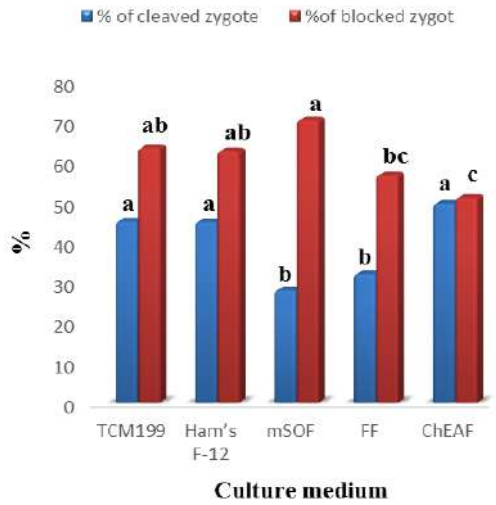

Figure 1. The effect of culture medium on cleavage and zygotic block rate

For somatic cell factor, the COEC resulted in high cleavage rate and low developmental block compared to other co culture cells. The effect of buffalo and goat OECs on buffalo's zygote development was studied by *Yadav et al. (1998*). They found that likewise buffalo OEC, the goat OEC resulted in improved

cleavage rate and decreased embryonic block which is in accordance with our study. The result of this work is also in accordance with *Romar et al. (2001)* and *Mugnier et al. (2009*) who showed that maturation of mare oocyte with oviductal cells resulted in more fertilization rate than culturing without oviductal epithelial cells. The positive effect of oocyte co-incubation with OECs on cleavage rate could be mediated by at least three mechanism: 1) increasing sperm lifetime; 2) decreasing polispermy; and 3) supplementing benefic materials to cleavage (*Mermillod et al., 1993; Smith, 1998; Coy et al., 2008*). The sperm lifetime is increased by temporary attaching to OECs in both in vivo and in vitro (*Smith, 1998*). It also has been demonstrated that ZP network which contains oviductal glycoproteins and other elements and surrounds oocytes is stabilized by binding of glycosaminoglycans (GAGs). GAGs decrease polispermy via increasing resistance to sperm penetration by modification of ZP solubility (*Coy et al., 2008*). Moreover, the low molecular weight (~10 kDa) of protein (proteins) which is present in oviduct cell cultures, supports early cleavage to 5-8 cell stage (*Mermillod et al., 1993*). In contrast to COEC, the lower cleavage was found in granulosa cells co-culturing that was in agreement with *Fassi-Fihri et al. (1991)* who reported that fertilization and cleavage rate were not affected by preovulatory follicles' granulosa cells.

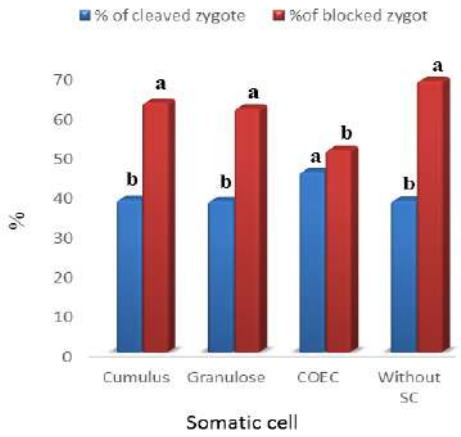

Figure 2. The effect of somatic cells on cleavage and developmental block rate

This study also demonstrated that developmental block rate was affected by medium, especially reduced in ChEAF medium. It was revealed that developmental block of pre-implantation embryos were affected by culturing media (*Bağış et al., 2001*). Embryo culture ingredient such as inorganic phosphate

and/or glucose have a detrimental effect on the development of early embryos (*Matsumoto and Sugawara, 1998*).

The concurrence of developmental block with genomic transition from maternal to embryonic genomes, suggests that it might be occurred by inability to overcome the chromatin repression or active transcription of important developmental genes or it is reaction to injuries caused by environmental conditions (*Meirelles et al., 2004*). However, the maternal mRNA and proteins are critical to early developmental stage of zygote (*Patel et al., 2013*). The developmental promoting activity of ChEAF may come from its ingredients that are rich of proteins, peptides, lipids, enzymes, hormones, growth factors (*Cho et al., 2007; Underwood et al., 2005*) such as TGF-β1 (*Mirzajani et al., 2011*), IGF-I (*Mcmurtry and Brocht, 1997*) and other materials that are useful for embryo development. It is demonstrated that TGF-β1, IGF-I (*Neira et al., 2010*), TGFα (*Larson et al., 1992*) increase blastocyst production.

Conclusion

The results of this work provided an additional observation concerning IVEP in cattle. The co-culturing of cattle oocytes with COEC in ChEAF medium resulted in higher cleavage rate and lower early embryonic developmental block. This study also revealed that COEC and ChEAF have an embryotropic effect in our experimental condition. Moreover, it is demonstrated that supplementation of co-culturing medium with 50% ChEAF not only supports oocyte and zygote but also supports somatic cells.

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Blokiranje razvoja zigota – uticaj stanja kulture

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Rezime

Uprkos napretku u *in vitro* proizvodnji embriona, blokiranje razvoja embriona se još uvek dešava u dosta zigota. Nedostatak razumevanja

mehanizma razvojnog bloka i njegovog odnosa sa suboptimalnim IVEP uslovima nas je ohrabrio da istražimo uticaj somatskih ćelija u ko-kulturi sa različitim medijima na ovu funkciju. Jajnici krava su prikupljeni iz lokalne klanice i prebačeni u laboratoriju za prikupljanje kumulus kompleksa jajnih ćelije (COCs). Odabrani COCs su sazreli u pet medija (TCM199, ham's F12, mSOF, folikularna tečnosti i embrionova plodova voda pileta) na 3 monosloja somatskih ćelija (kumulus, granulose i epitelne ćelije jajovoda) i bez somatskih ćelija u faktorijalnom dizajnu u periodu od 24 časa pod mineralnim uljem. Nakon IVF, paušalni zigoti su gajeni u sopstvenom tretmanu koji je sazrevao. Stopa cepanje i blokiranja je zabeležena 2-3 dana i 8 dana PI, respektivno. Medijum za kultivisanje koji sadrži ChEAF (embrionova amniotska tečnost pileta - chick embryonic amnion fluid) rezultiralo u najvećoj stopi cepanja (49.50±11.51), i najmanje blokiranih zigota (51.00±13.04) u odnosu na druge medije ($p<0,001$) i epitelne ćelije jajovoda krava (COECs) iz faktora somatskih ćelija su pokazala najveću stopu razdvajanja (45.31±12.96), a najmanje blokiranih zigota (50.85±14.24) iz drugih somatskih ćelija ($p<0,001$). Zigoti u ko-kulturi COECs u ChEAF medijumu su se značajno ($p<0,001$) razlikovali od ostalih tretmana u pogledu stope cepanja (57.54±10.36) i smanjenje blokiranja zigotskog razvoja (41.31±5.98). U zaključku, razvoj embriona se nalazi pod uticajem in vitro uslova iu ovoj studiji ChEAF sa epitelnim ćelijama kravljeg jajovoda je rezultiralo u manjem blokiranju razvoja.

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INFLUENCE OF PrP GENOTYPE ON MILK PRODUCTION TRAITS IN SLOVENIAN DAIRY SHEEP

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Original scientific paper

Abstract: The aim of this work was to analyse the association between PrP genotypes and milk production traits. A total of 7733 ewes with 23364 lactations data were used. Milk yield, milk fat and protein yield, milk fat and protein percentage from Bovec sheep, Improved Bovec sheep and Istrian pramenka were analysed. All genotypes were divided into five NSP types, based on National Srapie Plan. The most frequent NSP type was NSP3 at 61.90 %, followed by NSP2 (30.39 %), NSP1 (6.01 %) and NSP5 (1.24 %). NSP4 type was less than 1 %. No significant differences between NSP types and milk yield or milk fat and protein yield were observed. Milk fat percentage and milk protein percentage significantly increased from NSP1 type to NSP4 type , and then decreased in NSP5 to almost the same level as in NSP1 ($P<0.001$). These results should be considered when making further strategies for ewes-genotyping program to prevent possible decreasing of milk production traits in analyzed Slovenian dairy sheep.

Key words: PrP genotype, milk production, sheep breed, scrapie

Introduction

Scrapie is one of the transmissible spongiform encephalopathies, which also include Creutzfeld-Jakob disease in humans and bovine spongiphorm encephalopathy in cattle. Scrapie is also known as prion disease of sheep and goat. It is associated with polymorphisms in the prion protein (PrP) gene (Prusiner, 1998). Up to 5 scrapie haplotypes have been described according to amino acids transcribed at codons 136, 154, and 171 (ARR, ARQ, ARH, AHQ, and VRQ*) (Belt et al., 1995).* Those five haplotypes are related with resistance to scrapie. In general, it is accepted that mutations coding for A_{136} and R_{171} confer higher resistance, and those for V_{136} and Q_{171} render animals more susceptible to scrapie, while polymorphisms in codon 154 are considered of minor importance. As a result, animals of the ARR/ARR homozygous genotype are considered highly

resistant and those of the VRQ/VRQ genotype highly susceptible to clinical scrapie. *Alexander et al. (2005)* reported that the degree of susceptibility to scrapie in sheep is also breed depended.

The evidence for differing degrees of genetic resistance to scrapie for different PrP genotypes forms the basis of National Scrapie eradication Plan (NSP). Moreover, the possible relationships between scrapie in sheep and BSE in cattle have lead the European Union to determine the establishment of breeding programs aimed at selection for resistance to scrapie in sheep breeds in all member-states *(European Commission, 2003)*. Those NSP are now underway in some European countries with a broad aim to increase the frequency of resistant ARR haplotype in the national flocks whilst at the same time reduce the frequency of the VRQ haplotype. The breeding program strategies to achieving the aim of NSP's varies between countries. More rigorous eradication scrapie plans only allows mating using homozygous ARR rams, for example Netherlands NSP *(Windig et al., 2004)*. Less rigorous NSP allows mating using heterozygous rams carrying ARR, ARH, AHQ or ARQ *(Defra, 2004)*. Consequently, both eradication plans will eradicate the VRQ haplotype and increase the frequency of the ARR haplotype, although the Netherlands plan is more likely to increase the ARR haplotype frequency in a shorter time period but with greater threat to their genetic base.

From 2006, the eradication scrapie plan has been included into Slovenian breeding programs for all sheep breeds, to increase the scrapie resistance in national sheep flocks. The Slovenian eradication scrapie plan is based on ram- and ewe-genotyping program. On the basis of the results of the PrP genotypes the scrapie eradication plan is performed but little is known about potential correlated effects of PrP genotype on milk production traits in Slovenian dairy sheep. So far scarce studies on production or reproduction traits associated with PrP genotype have been reported *(Casellas et al., 2007; Ioannides et al., 2009; Vitezica et al., 2013; Lipsky et al., 2008; Alexander et al., 2005)*. The results of those studies indicating that there are no associations of the PrP genotype with dairy traits and minor with reproductive traits.

The aim of this work was to investigate whether there was a difference in milk yield per lactation, fat and protein yield and fat and protein percentage associated with the ewe's NSP type.

Material and Methods

In this study 7733 ewes of three breeds (Bovec sheep, Improved Bovec sheep and Istrian pramenka) belonging to 49 flocks were obtained from the Data Base of Slovenian national sheep breeding program. All ewes had been PrP genotyped through the regularly annual national ewes-genotyping program. Milk

yield, fat and protein yield, fat and protein percentage were recorded according to official test-day method of the selection scheme from 1995 through 2014. These measurements included 23364 records (lactation performance) for milk yield per lactation, milk fat and protein yield, and milk fat and protein percentage. The data collection included also pedigrees, PrP genotype and NSP type from three dairy sheep.

Data were analysed using the GLM procedure (General Linear Model) in SAS/STAT (SAS Institute Inc., 2011). The log-linear model (1) for milk yield, milk fat and protein yield, milk fat and protein percentage (Y_{ijklm}) considered NSP type (G_i) (i=1-5), breed (B_j) (j=1-3), flock (farm management) (F_k) (k=1-49) and year of lambing (L_l) (l=1-22) as fixed effect. The linear regression coefficient (b) of the successive lactation was included in the model.

$$Y_{ijklm} = \mu + G_i + B_j + F_k + L_l + b\left(X_{ijklm} - \overline{X}\right) + e_{ijklm} \qquad \text{Model (1)}$$

To obtain a better distribution of frequencies between comparison PrP genotypes, all genotypes were divided into 5 types, based on NSP. The NSP1 type comprised scrapie-resistant animals or homozygous ARR/ARR. The NSP2 type comprised the nonsusceptible ewes (ARR/XXX). The NSP3 type comprised neutral animals (AHQ/AHQ, AHQ/ARH, AHQ/ARQ, ARH/ARH, ARH/ARQ, ARQ/ARQ, ARK/ARK). The ARR/VRQ animals belonged to the NSP4 type. Finally, the highly susceptible VRQ/VRQ animals comprised NSP5 type.

Results and Discussion

All major sheep breeds currently used for milk production in Slovenia were included in this study, with a total of 3 breeds represented. The observed population structure associated with NSP type frequency is presented in Table 1.

Table 1. Frequency of PrP genotypes divided into NSP types by breed

	Bovec sheep		Improved Bovec sheep		Istrian pramenka		Total	
NSP type	N	%	N	%	N	%	N	%
1	178	3.68	71	5.80	216	12.91	465	6.01
2	1242	25.69	420	34.28	685	40.94	2347	30.39
3	3361	69.51	720	58.77	706	42.20	4787	61.90
4	12	0.25	4	0.33	22	1.32	38	0.49
5	42	0.87	10	0.82	44	2.63	96	1.24
Total	4835	100	1225	100	1673	100	7733	100

The most frequent NSP type was NSP3 at 61.90 %, followed by NSP2 type (30.39 %). There were only 6.01 % of ewes carrying ARR/ARR alleles. The frequency of NSP4 type was less than 1 %. The frequency of NSP5 was 1.24 %. So-called wild-type allele in sheep (ARQ) was the most frequent genetic variant in those three breeds with a frequency of 63.14 %, followed by the haplotype ARR, at 32.91 % (data not shown). The frequency of the most susceptible haplotype, VRQ, was small but presented at 1 % (data not shown). The frequencies observed in Bovec sheep, Improved Bovec sheep and Istrian pramenka sheep were similar to those of other breeds analysed, as Churra *(Álvarez et al., 2006)*.

The breed of the ewes, lambing season, flock and successive lactation shown highly significance influences in all observed traits (P<0.0001), but not presented here. The effect of PrP genotype is shown in Table 2.

Table 2. Lactation milk yield, lactation fat and protein content, lactation fat and protein percentage and litter size in milk breed ewes according NSP type (LS Means ± SE)

	NSP type					
	1 N=1295	2 N=6896	3 N=14692	4 N=130	5 N=351	Sig.
Litter size	1.33 ± 0.04^{ab}	1.30 ± 0.03^{a}	1.30 ± 0.03^{a}	1.36 ± 0.05^{b}	1.32 ± 0.04^{ab}	*
Milk yield (kg)	169 ± 6.7	164 ± 6.3	165 ± 6.3	172 ± 9.5	167 ± 7.6	ns
Milk fat (kg)	10.33 ± 0.44	10.23 ± 0.41	10.24 ± 0.41	11.07 ± 0.62	10.20 ± 0.49	ns
Milk fat (%)	6.26 ± 0.08^{a}	6.37 ± 0.07^{b}	6.35 ± 0.07^{b}	6.52 ± 0.11^{c}	6.28 ± 0.09^{a}	**
Milk protein (kg)	8.94 ± 0.37	8.74 ± 0.35	8.76 ± 0.35	9.34 ± 0.52	8.65 ± 0.42	ns
Milk protein (%)	5.34 ± 0.05^{a}	5.39 ± 0.04^{b}	5.39 ± 0.04c	5.45 ± 0.07^{b}	5.30 ± 0.05^{a}	**

ns: P>0.05, *P<0.05, **P<0.001, ***P<0.0001
^{a,b,c} least squares means within a row with different letters are significantly different

The production traits as milk yield, milk fat and protein yield were not affected by NSP type. These results confirmed with the results of *Álvarez et al. (2006), Ioannides et al. (2009), and Vitezica et al. (2013),* where no significant differences between PrP genotypes for milk yield and milk quality traits were found. However, in our study, milk fat percentage and milk protein percentage differ significantly between NSP types (P<0.001). Milk fat percentage and milk protein percentage significantly increased from NSP1 to NSP4 type, and then decreased in NSP5 to almost the same level as in NSP1. However, the differences in milk fat and protein percentage were probably due to differences in milk yield, which also differ by NSP, but the differences were not significant. *Álvarez et al. (2006)* found small differences among ARQ/ARQ, AHQ/XXX, and ARH/XXX,

with slightly negative differences (but not significant) between the wild type and the most resistant genotypes.

Nevertheless, intense selection for the PrP genotype alone could have undesirable consequences in terms of inbreeding, and correlated responses in production and adaptation traits (*Gama et al., 2006*), so breeding strategies for scrapie eradication program for analyzed breeds need to be planed carefully in the future.

Conclusions

The results of this study lead to the hypothesis that increasing resistance to scrapie in Slovenian dairy sheep population could have minor adverse effect on selection for fat and protein percentage in milk. These results should be considered when making further strategies for ewes-genotyping program to prevent possible decreasing of milk production traits and the most important to prevent possible decreasing of genetic variation.

Uticaj PrP genotipa na proizvodne osobine slovenačkih mlečnih ovaca

A. Cividini

Rezime

Skrapi (engl. – Scrapie; bolest češanja, svrabež) je prionska bolest koja utiče na ovce i koze. Osetljivost na skrapi je povezana sa polimorfizmom u prion proteinu (PrP) gena ovaca. Evropska unija je realizovala Nacionalne planove za skrapi - National Scrapie Plans (NSP) u svim zemljama, oslanjajući se na selektivni uzgoj grla otpornih na skrapi. Od 2006. godine, NSP je uvršten u slovenačke odgajivačke programe za sve rasa ovaca. Na osnovu NSPa, PrP genotipovi su klasifikovani u pet NSP tipova kako bi se dobila bolja raspodela frekvencija između PrP genotipova za poređenje. Tip NSP1 je životinja otporna skrapi ili homozigotna ARR/ARR. NSP2 je bila sastavljena od ovaca koje nisu podložne/prijemčive (ARR/XXX). NSP3 se sastoji od neutralnih životinje. ARR/VRQ životinje su pripadale tipu NSP4. Visoko prijemčive životinje su uključivale sve VRQ nosioce u NSP5 tipu.

Analiziran je odnos između PrP genotipa klasa i proizvodnih osobina bovec ovaca, oplemenjenih bovec ovaca i istarske pramenke. U ispitivanju je korišćeno ukupno 7733 ovaca sa podacima za 23364 laktacija. Prinos mleka, prinos

mlečne masti i proteina, i sadržaj mlečne masti su analizirani sa GLM postupkom u SAS/STAT (SAS Institute Inc., 2011). Nisu uočene značajne razlike između vrste NSP i prinosa mleka, ili mlečne masti i proteina. Mlečna mast i sadržaj proteina značajno je povećan od NSP1 do NSP4 (P<0,001). Međutim, veći sadržaj mlečne masti i proteina u NSP4 je verovatno zbog visokog prinosa mleka u NSP4 (ne značajno). Kao posledica toga, skrapi genotipizacija može biti dalje uključena u strategiju uzgoja kao kriterijum u selekciji, ali izbegavajući mogući rizik od smanjenja proizvodnih osobine slovenačkom muznih grla.

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THE EFFECT OF WOLF AND BEAR ATTACKS ON GROWTH IN LAMBS

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Abstract: Wolf and bear attacks are not stressful only for the attacked sheep, but also for those witnessing the attack. The consequences can be reflected in reduced production. To test this assumption we evaluated growth rates of lambs in attacked Slovenian sheep flocks. Data of wolf and bear attacks in the period 2008-2012 were compared with performance of lambs present in the attacked flock during this period. Lambs witnessed up to three wolf attacks and one bear attack, and 191 lambs were present in a flock when at least one attack of a large predator occurred. Number of wolf attacks negatively affected lambs' growth. Lambs witnessing more than one wolf attack grew significantly slower (44 g/day) than lambs that experienced only one attack. Additionally, negative regression coefficient revealed a long term negative effect of wolf attack on lambs' daily gain. It decreased for 0.66 g/day if the period between the first attack and the end of fattening period (at 60±10 days) increased for one day. Therefore, if the flock was attacked early in lamb's life, its daily gain was negatively influenced during its whole growth period, indicating prolonged stress which inhibited growth. Surprisingly, in bear regression coefficient was positive (0.66 g/day), indicating short term stress, meaning that if the attack happens early in lamb's life, the lamb recovers from stress.

Key words: sheep, daily gain, wolf, bear, attacks

Introduction

In Slovenia, sheep killed by wolves and bears are recognised by the state as economic loss. However, the problem of severe stress in relation to animal welfare is usually ignored. The ignored group are also the animals in the attacked flock which can be injured or are mere witnesses of attack or repeating attacks. The stress they experience compromises their welfare and presumably also their production.

Farm animals are selected for high production which can therefore persist also in the environments which are not following animal welfare requests. However, numerous studies show negative effects of stress on the performance of farm animals, where unpredictability and uncontrollability of the events play an important role (*e.g. Koolhaas et al., 2011*). Various stress factors can change their immune function, increase susceptibility to disease, decrease feed intake and rumination, reduce fertility, inhibit oxytocin release (*as listed by FAWEC, 2013*), reduce fitness of the animals, productivity and quality of their products (*e.g. Gebregeziabhear and Ameha, 2015*).

The occurrence of attacks cannot be predicted by the sheep, there can be only one attack on the flock or several, repeated in very variable time intervals. The attacks are also uncontrollable, because usually the sheep cannot retreat from dangerous situation. Therefore the animals are experiencing chronic stress. *Dwyer and Bornett* (*2004*) reviewed many negative effects of chronic stress on sheep, e.g. on reproduction, meat quality, feeding behaviour, lameness, parasitism and so forth. As for the effect on growth rate, the results they had examined were inconclusive. However, *Kolb et al.* (*2000*) found that prolonged stress inhibits sheep growth and reproduction.

Slovenian sheep breeders repeatedly observe production declines in sheep flocks attacked by wolves and bears (like increased abortion rate, decreased weight gain or lower milk production). To test these assumptions, we compared the dates of reported wolf and bear attacks with the daily gain of lambs witnessing the attack for the period of five years.

Material and methods

The data set combines data of wolf and bear attacks in the period from year 2008 to 2012 with the performance of lambs present in the attacked flock during this period (the lambs were born between October 2007 and November 2012). These data originated from two official databases. The dates of wolf and bear attacks were collected from the database of damage caused by protected wild species at Slovenian Environment Agency, while lambs' daily gain was calculated on the basis of data recorded as part of the Slovenian national sheep breeding program at the Department of Animal Science at Biotechnical faculty, University of Ljubljana. Lambs were weighed two to three days after birth and at the end of fattening period at the age of 60±10 days.

In the period from 2008 to 2012 lambs witnessed up to three wolf attacks and one bear attack (Table 1). 191 lambs were present in a flock when at least one attack of large predator occurred and more than 3/5 of them were present at wolf's attacks.

Table 1. Number of lambs witnessing one, two or three attacks, respectively

Number of attacks	Wolf	Bear
1	101	70
2	16	
3	4	
Total	121	70

Statistical evaluation was performed with R programming language, ver.3.1.1 *(R core team, 2014)*, packages car, ver. 2.0-21 (*Fox and Weisberg, 2011*) and lsmeans, ver. 2.13 *(Lenth and Hervé, 2014)*. For the purpose of statistical analysis the data of two and three wolf attacks were summed up. Daily gain of lambs ($Y_{ijklmno}$) witnessing the attack of wolf was studied with the following statistical model:

$$Y_{ijklmno} = \mu + P_j + L_k + B_l + S_m + O_n + N_i + b_1\left(A_{ijklmno} - \overline{A}\right) + \\ + b_2\left(E_{ijklmno} - \overline{E}\right) + e_{ijklmno} \qquad <1>$$

where μ is the mean of the model, P_j is the parity number, L_k litter size (1, >1), B_l sheep breed (Jezersko-Solčava, Improved Jezersko-Solčava sheep), S_m sex of the lamb, O_n type of rearing (conventional, ecological), N_i is the number of attacks (1, >1), b_1 is regression coefficient of fattening period duration on daily gain and b_2 is regression coefficient of time period between the first attack on the flock and the weighing at the end of fattening period on daily gain.

Since in bear no lamb witnessed more than one attack, the statistical model for studying their daily gain is a bit different from the model <1>:

$$Y_{ijklmn} = \mu + P_i + L_j + S_k + O_l + b_1\left(A_{ijklmn} - \overline{A}\right) + b_2\left(E_{ijklmn} - \overline{E}\right) + e_{ijklmn} \qquad <2>$$

The meaning of letters in the model <2> is the same as in the model <1>.

Results and discussion

In flocks attacked by wolf the lambs' daily gain was influenced by parity, breed, number of attacks, fattening period in days and number of days between the first attack and weighing at the end of fattening period (Table 2). In lambs witnessing the attack of bear the daily gain was influenced by type of rearing, fattening period and the period between the first attack and the end of fattening period. Further, only the results for the number of attacks and the period between

the first attack and weighing at the end of fattening period are presented in details (Table 3).

Table 2. Results of analysis of variance for lambs' daily gain according to models <1> (wolf) and <2> (bear)

Influence	Wolf	Bear
Parity (P_i)	0.02839	0.08012
Litter size (L_k)	0.76966	0.66611
Breed (B_l)	0.02430	
Sex of the lamb (S_m)	0.66481	0.31243
Type of rearing (O_n)	0.69058	<0.00001
Number of attacks (N_i)	0.00041	
Fattening period (reg. b_1)	<0.00001	0.02840
Period from 1st attack and final weigh. (reg. b_2)	0.00380	0.01695

Number of wolf attacks negatively affected lambs' growth (Table 3). Lambs witnessing more than one wolf attack grew significantly slower, that is for 44 g/day, compared to lambs that experienced only one attack. Additionally, regression coefficient of the period between the first attack and weighing at the end of fattening period revealed that wolf attack had a long term negative effect on lambs' daily gain. Daily gain decreased for 0.66 g/day if the period between the first attack and the end of fattening period increased for one day. So, if the flock was attacked early in lamb's life its daily gain was negatively influenced during its whole growth period. According to the results of *Kolb et al.* (*2000*) we can conclude, that wolf attack, even if there is only one in lambs life, represents prolonged stress, which inhibits their growth. However, as reviewed by *Dwyer and Bornett (2004)* the effect of stress on growth rate in lambs might not be only direct via physiological stress response, but also secondary, like through decreased feed intake. Wolf attack probably does not affect only lambs, but also their mothers. It was found out, that growth in lambs can be affected also by stressed mothers, through their reduced milk production *(Sevi et al., 2001;Caroprese et al., 2010)* and milk flow *(Tančin et al., 2015)*, and by lower milk quality *(Sevi et al., 2001)*. Stress can change also their maternal behaviour, like more frequent udder refusal and reduced following behaviour as observed by *Lv et al. (2015)*.

Table 3. LSM ± standard error of lambs' daily gain (g/day) according to number of attacks and regression coefficient of period between the first attack and the final weighing on daily gain (b_2)

	Wolf	Bear
Number of attacks:		
1	228±6[a]	
> 1	184±11[b]	
Period from 1st attack and final weigh. (reg. b_2)	- 0.66±0.22	0.66±0.26
[a, b] The numbers with different superscript within a column differ significantly		

In the analysed period between the years 2008 and 2012 there was no repeated bear attacks as there were in wolves, therefore a direct comparison of bear and wolf attack results is not possible. In bear, the effect of the attack on lambs' daily gain was not the same as in wolf although presuming that for the witnessing animals the bear attack is as stressful as wolf attack, we would expect similar results. However, according to the positive regression coefficient of the period between the first bear attack and lambs weighing at the end of fattening period (Table 3), we cannot talk about the long term negative effect. The increment of daily gain for 0.66 g/day is indicating that bear attack is probably not presenting prolonged stress as does the wolf attack, but rather a short term stress. This means that if the attack happens early in lamb's life, the lamb recovers from stress.

Conclusions

In flocks attacked either by wolf or bear, the attack significantly influenced the growth of lambs witnessing the attack. Wolf's attack had a long term negative effect on lambs' daily gain. The younger was the lamb witnessing the attack, the smaller was its daily gain. In wolf, repeated attacks also significantly contributed to slower lambs' growth. On the contrary, bear attack seems to have a rather short term effect on lambs' growth, giving lambs witnessing the attack in their early age a chance to recover.

Uticaj napada vukova i medveda na rast jagnjadi

M. Kastelic, N. Siard, M. Fučko , D. Jordan

Rezime

U Sloveniji, ovce ubijene od strane vukova i medveda su priznate od strane države kao ekonomski gubitak. Međutim, napadi nisu stresni samo za napadnute

ovce, već i za one koji su svedoci napada. Posledice se ne ogledaju samo na njihovu dobrobit, već i na njihovu proizvodnju. Da bi testirali ovu pretpostavku, ispitali smo stope rasta jagnjadi u napadnutim slovenačkim stadima ovaca. Podaci o napadima vukova i medveda u periodu 2008-2012 su poređeni sa performansama jagnjadi prisutnih u napadnutom stadu tokom ovog perioda. Jagnjad su bili svedoci do tri napada vukova i jednog napada medveda, a 191 jagnje je bilo prisutno u jatu u trenutku barem jednog napada velikog predatora i više od 3/5 od njih su bili prisutni prilikom napada vukova. Broj napada vukova negativno je uticao na rast jagnjadi. Jagnjad prisutna prilikom više od jednog napad vukova pokazuju značajno sporiji porast (44g/dan) nego jagnjad koja je doživela samo jedan napad. Osim toga, negativan koeficijent regresije otkrio je dugoročni negativan efekat napada vukova na dnevni prirast jagnjadi. Dnevni prirast jagnjadi je bio manji za 0,66 g/dan ako je period između prvog napada i kraja perioda tova (na 60 ± 10 dana) povećan za jedan dan. Dakle, ako je stado napadnuto rano u životu jagnjeta, njegov dnevni prirast je bio pod negativnim uticajem napada tokom celog perioda rasta, što ukazuje na produženi stres koji inhibira rast. Iako direktno poređenje između napada vukova i medveda nije bilo moguće, jer nije bilo ponovljenih napada medveda, i dalje je iznenađujuće da je pretpostavka da su napadi vukova i medveda podjednako stresna nije potvrđena, jer je u slučaju medveda koeficijent regresije bio pozitivan (0,66 g/dan), što ukazuje na kratkoročni stres, što znači da ako se napad desio rano u životu, jagnje će se oporaviti.

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PSEUDOMONAS AERUGINOSA IN SEMEN BOARS AND POSSIBLE SOURCES OF INFECTION

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Original scientific paper

Abstract: The causes of reproductive disorders that occur in swine production are complex and are often multicausality. Because of their complexity it is often not easy to determine what is the primary cause of the disorder and for this reason it will be necessary to analyze the critical points of production. In swine production sterility can be caused by reproductive disorders of boars as well as sows. The estimation of semen quality is very important issue for solving a reproductive problems caused by boars. Semen quality includes various qualitative and quantitative parameters, among which microbiological monitoring is, certainly, an important place. In the process of artificial insemination, when used diluted boar semen, it is possible that there is a problem of pregnancy as a result of bacterial contamination of native semen. For these reasons, it seems that the microbiological monitoring of native semen is a good way of prevention of subsequent reproductive problems. *Pseudomonas aeruginosa* is the opportunistic pathogen, whose presence in boar semen may lead to changes in semen quality. Considering the very wide the prevalence of these bacteria in nature is not easy to determine the route of transmission or the source of infection of boars with *Pseudomonas aeruginosa* in the facilities. The subject of our study was the microbiological analysis of native boar semen and samples of water from the drinkers in the objects of growing these animals.Our goal is to determine whether the samples of semen contain *Pseudomonas aeruginosa* as well as to determine whether the same microorganisms are present in the water samples from the drinkers. During a one year a total of 79 samples of native semen boars are examined. Total of 29 (36.70%) of the samples have been positive for the presence of *Pseudomonas aeruginosa*. The presence of these bacteria was determined in the samples with a small total number of bacteria (100-1000cfu / ml) as well as in the samples with > 10.000cfu / ml. In the water samples it is confirmed presence of the same microorganism. These findings indicate a real risk of changes of boar semen quality and the possibility for reproductive disorders in sows. Detection of *Pseudomonas aeruginosa* in water from drinkers could indicate a potential source of boar infection with this bacteria.

Keywords: boar, native seeds, water, Pseudomonas aeruginosa

Introduction

The most important segment of the modern livestock production is to ensure optimal reproductive conditions. The possibility of obtaining healthy progeny while retaining all production characteristics of animal exploitation is a perspective of the economic of production. In order to achieve a quality of reproductive cycles it is important to monitor the health condition of female reproductive organs as well as male individuals. In pig production a quality of boar sperms are the basis for quality reproductive cycle. The artificial insemination is widespread used in swine production (*Maes et al. 2008*) due this it is very important to ensure quality semen. In order to obtain the quality sperm it is necessary to perform different types of tests (*Milovanović et al. 2015*).

In assessing the quality of semen an important role has a sperm contamination by microorganisms. This fact is based on the circumstances that the collection of semen during ejaculation is not done in sterile conditions (*Úbeda et al. 2013*). Certainly, that besides of bacterial species that can be found in the semen, the level of microbiological contamination is also the importance for the quality of the sperm (*Sepúlvedaa et al. 2014*). As we know, the semen for artificial insemination is being diluted and this process decreases the number of bacteria and also the used antibiotics in the extended can suppress their growth. According to the literature in collected sperm there is a possiblity for isolation a different types of bacteria: from the family *Enterobacteriaceae - Serratia, Klebsiella, Providence, Morganella, Proteus* and *Escherichia* coli and *Staphylococcus species*, *Streptococcus* species, *Pseudomonas* and other (*Úbeda et al. 2013*; *Althouse and Lu, 2005; Baracaldo, 2008*). The presence of the certain types of bacteria in the sperm may be explained by the infection of urogenital tract by bacteria which originated from faeces (*Maroto et al. 2010; Althouse and. Lu, 2005*), and some, such as Gram-positive bacteria can be origin from the skin or respiratory tract. *Pseudomonas species* are opportunistic pathogens widespread in nature (earth and water) (*Stojanov et al. 2013*).

Finding these bacteria in water samples taken from drinkers that supply animals to the facilities can point to a potential source of infection (*Stojanov et al. 2014*). The presence of *Pseudomonas aeruginosa* in water can permanently contaminate the facility and especially the area around the drinker, which contributes to the occurrence of infection or reinfection. For these reasons, the subject of our research is the microbiological analysis of native boar sperm and samples of water from the drinkers in the objects of growing these animals. Our

goal is to determine whether the samples of semen contain *Pseudomonas aeruginosa* as well as to determine whether the same microorganisms are present in water samples from the drinkers.

Material and Methods

Tests were carried out on 79 samples of native semen boars. Samples were collected from four different center which are located within the pig farms. Semen quality is assessed with CASA (computer assisted sperm analyse), determing the number of total and progressive motility and sperm speed parameters. In case of low sperm quality, row and diluted semen samples were subjected to microbiological analysis. Semen was collected by manual fixation with gloved-hand technique. Pre-sperm fraction was discarded and 20 ml of semen was taken directly in the sterile plastic pots for bacterialogical survey. In total, 4 water sample from the drinkers were examined. The samples were comprehended water (Samples of water were taken, from a farms where boars are used for semen production as well as samples from farms that boars are not in exploatation. Identification of bacterial contamination in 79 samples of sperm is done by plating semen in the blood agar, McConkey agar (Noack) and incubated at 37^0C for 24 hours. In order to determine the presence of yeast and mold samples were inoculated on Sabouraud agar (Noack) incubating at 24^0C for 96 h. The samples were inoculated on blood agar plates and incubated under anaerobic conditions for 48 h. To determine the number of bacteria in the semen, we used the plate count agar (Noack) which was inoculated with 1 ml diluted sample (1/10, 1/100 and 1/1000). The presence Gram positive or negative microorganisms has been done by microscopic as well do those bacteria belong to rods or cocci. After catalase, oxidase and OF tests the identification of bacteria has been done by determining the biochemical characteristics of the isolates. (*Quinn et al. 1998*). For the isolation and identification of bacteria from the taken water samples we used methods for bacteriological, virological, biological and parasitological examination of drinking water, Appendix III present Regulation on the manner of taking samples and method for laboratory analysis of drinking water (*Official Gazette, 1987*).

Results and discussion

Semen of boars is a good medium for the growth and multiplication of bacteria. Although some parts of the reproductive tract of boars considered as free from bacteria, after collection of ejaculated semen it can contain bacteria from 10^4 to 10^5 / ml (*Sone 1990*), but according to some authors, the number of bacteria can be from 10^2 to 10^6 . Mostly present bacteria belonging to the group of Gram

negative and particularly the group of glucose non fermentative bacteria (*Quinn et al. 1998*, *Baracaldo 2008*). It should noted that, the mentioned bacteria does not represent pathogens which, via artificial insemination, can be transmitted to sows and causing disease. Despite of possibility that some important pathogens can be transmitted by sperm (*Brucellosis, Chlamydia sp, Leptospirosis,* and *Tuberculosis Mycoplasma* sp) (*Maes et al. 2008*) the mentioned bacteria can only contribute to the reduction of sperm quality. Their presence can be of different origin (hygiene facilities, staff employed, preputial fluid, feces, skin and hair) (*Althouse and Lu 2005*; *De Grau et al., 2006*).

Table 1 contains information on the types and findings of certain types of bacteria in the semen. All examined samples (79) of native boar sperm contained different bacterial species and the number of bacteria ranged from 100 to> 10.000cfu / ml.

Table 1. Bacterial isolates of boars semen

Bacterial genus and species	The presence of bacteria (%)
Staphylococcus sp. (coagulase-negative species)	23.45
Staphylococcus aureus	3.10
β hemolytic streptococci	2.30
Enterococcus sp.	9.45
Bacillus sp.	19.20
E. coli	21.40
Enterobacter sp.	22.60
Micrococcus sp	18.90
Citrobacter sp.	8.10
Pseudomonas aeruginosa	36.70
Acinetobacter spp.	5.40
Aeromonas sp.	1.40

Our results show that the presence of certain types of bacteria corresponds to the findings of which were obtained by other authors. In the article of *Martin et al. (2010)* the researchers found that the most frequently isolated bacteria from semen is *Escherichia coli* (79%), however significantly presence of *Proteus sp*, *Serratia sp*, *Enterobacter sp*., *Klebsiella sp*., *Staphylococcus sp*., *Streptococcus sp*. and *Pseudomonas sp.* (41.33 to 7.33%). The authors reported in the paper (*Althouse and Lu, 2005*), that the most frequent bacteria in the extended sperm of boars were *Enterococcus sp.* (20.5%), *Stenotrophomonas sp.* (15.4%), *Alcaligenes sp.* and *Serratia* sp. (10.3%), *Acinetobacter sp*. (7.7%), *E. coli* (6.4%), *Pseudomonas spp*. (6.4%) and 24.0% of the other bacteria. According to the author Ubeda et al. (*Úbeda et al. 2013*) bacteria from the family *Enterobacteriaceae* accounted for 40.68% of the isolates of native semen boars. In our study, it can be seen that the

Pseudomonas aeruginosa (36.70%) represented by far the most frequently bacterial species in native sperm of boars. Semen quality certainly depends on the presence of bacteria and their number in the semen. In the paper Yaniz et al. **(***Yániz et al. 2010*) authors examined the influence of bacterial flora for sperm quality during storage at 15^0C. Their studies have shown that particular bacteria from the family *Enterobacteriaceae* have a huge influence on semen quality during the storage.

Microbiological testing of water samples from drinkers showed the presence of a large number of different species of bacteria. In table 2 there are the results of tests of water samples.

Microbiological water monitoring showed that in the examined samples there can be various opportunistic pathogens such as *Pseudomonas spp*., *Aeromonas sp*., *Flavobacterium sp*. Finding these bacterias can be related to a specific role of water supply system on the farm.

Table 2 Bacteria isolated from water samples

Bacterial genus and species	Drinking trough	Bacterial genus and species	Drinking trough
Aeromonas spp.	+	*Staphylococcus sp.*	-
Aeromonas hydrophila	+	(coagulase-neagative species)	
Bacillus spp	-	*Citrobacter freundii*	-
Corynebacterium spp.	-	*Pseudomonas aeruginosa*	+
Enterococcus spp.	+	*Pseudomonas spp.*	+
Escherichia coli	+	*Enterobacter aerogenes*	+
α hemolytic streptococci	+	*Enterobacter sp.*	+
Micrococcus luteus	-	*Flavobacterium sp*	+

+ (presence of bacterial species), - (absence of bacterial species)

The research of authors (*Stojanov et al. 2015*) showed that, during the examination of water samples from different places of water supply system in pig farms, can be recognized a huge influence of the application of antibiotics through water supplying system as well as on microbiological structure of water. The treatment of a large number of animals are most easily done, per oral, by application of antibiotics through the water. This application of antibiotics, through some time period, leads to the selection of micro-organisms in the water, i.e., there will remain only the resistant strains in water. In this occasion, in the drinkers retained bacteria in longer period of time, that can contaminate the environment of facilities and animals. The presence of *Pseudomonas aeruginosa* in water corresponds to the findings of other authors who have confirmed the presence of these bacteria both in water and waste water from farms (*Lechevallier et al.1980; Stojanov et al. 2014*). Detection of *Pseudomonas aeruginosa* in samples of water from drinkers can be explained by the possibility of the survival of these bacteria in very unfavorable conditions (*Quin et al*, 1998), but also by the fact that during the

supplying animals with water, in drinkers can be retained particle of food and animal saliva which allows bacteria to survive.

Conclusion

Microbiological analysis of native boar sperm showed the presence of a large number of different species of bacteria. We confirmed the presence of the bacteria from the family *Enterobaactericeae*, *Staphylococcus sp*. and *Streptococcus sp*. Among the gram-negative glucose non fermented bacteria, *Pseudomonas aeruginosa* is dominated. This bacterium was present in 36.70% of samples of native sperm of boar. Due to decreased semen quality of boars it can be assumed that the isolated bacterial flora contribute to this phenomenon.

The tested water samples taken from the drinkers in the facilities, in which animals are grown, revealed the presence of different number of bacteria. We found in all samples *Pseudomonas aeruginosa*. This finding points to possible health problems that may arise as a result of the introduction of Pseudomonas aeruginosa across the water. Certainly, persistence of this microorganism in the drinker and its spread in the house can be a source of infection of the reproductive tract of boars. Monitoring of the presence of bacteria in the water supplying system and microbiological control of boar semen, as well as all necessary hygiene measures in facilities breeding animals represent an important segment for obtaining maximal results in swine reproduction.

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Nalaz *Pseudomonas Aeruginosa* u semenu nerasta i mogući izvori infekcije

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Rezime

Uzroci reproduktivnih poremećaja koji se javljaju u proizvodnji svinja su složeni i najčešće su multikauzalni. Zbog svoje kompleksnosti često nije lako utvrditi šta je primarni uzrok poremećaja i iz tih razloga neophodno je sve kritične

tačke proizvodnje analizirati. Sterilit, uzrokovan od strane nerasta izazvan lošim kvalitetom semena prioritet je u kontroli reproduktivnih smetnji u proizvodnji svinja.

Kvalitet semena obuhvata različite kvalitativne i kvantitativne parametre među kojim mikrobiološki monitornig svakako zauzima značajno mesto. U postupku veštačkog osemenjavanja krmača, kada se koristi razređena sperma nersta, moguće je da dođe do problema suprasnosti kao posledica bakterijske kontaminacije nativnog semena. Iz tih razloga čini se da je mikrobiološki monitoring nativnog semena dobar put preventive kasnijih reproduktivnih problema. *Pseudomonas aeruginosa* je oportuni patogen, čije prisustvo u semenu nerasta može dovesti do promene kvaliteta semena. Sa obzirom na veoma široku rasprotranjenost ove bakterije u prirodi nije lako utvrditi put prenošenja, odnosno izvor infekcije nerasta sa *Pseudomonas aeruginosa* u objektima.

Predmet našeg istraživanja je bila mikrobiološka analiza nativne sperme nerasta i uzoraka vode iz pojilica u objektima držanja ovih životinja. Cilj nam je da utvrdimo da li se u uzorcima semena nalazi *Pseudomonas aeruginosa* kao i da utvrdimo da li se isti mikrooganizam nalazi u uzorcim vode iz pojilica. Tokom jedne godine ukupno je pregledano 79 uzoraka nativnog semena nerasta. Ukupno 29 (36,70%) uzorka je bilo pozitivno na prisustvo *Pseudomonas aeruginosa*. Prisustvo ove bakterije je utvrđeno kako kod uzoraka sa malim ukupnim brojem bakterija (100-1000cfu/ml) tako i kod uzoraka sa >10.000cfu/ml. U uzorcima vode je potvrđeno prisutvo istog mikroorganizma. Ovakavi nalazi ukazuju na realnu opasnost promene kvaliteta semene nerasta i mogućnost reproduktivnih smetnji kod krmača. Nalaz *Pseudomonas aeruginosa* u vodi iz pojilica mogao bi ukazati na potencijalni izvor infekcije nerasta ovom bakterijom.

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POSTER SECTION I

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THE INFLUENCE OF THE FACTOR «LIVE WEIGHT AT THE FIRST CALVING» ON CONTROLLED SIGNS OF PRODUCTIVITY AND DURATION OF ECONOMIC USE OF COWS OF THE YAROSLAVL BREED AND ITS HYBRIDS WITH HOLSTEIN

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Original scientific paper

Abstract: The Yaroslavl region refers to high developed agricultural regions of the Russian Federation. The efficiency of leading dairy cattle breeding industry of the region depends on the level of milk production and on the duration of the economic use of animals. These indicators are not only biological, but also economic. It is necessary to create highly productive herds and to increase the duration of the economic use of animals and consequently lifetime productivity for the intensification of dairy farming of the region. Therefore, the aim of our research was to determine the influence of the factor «body weight at the first calving» on the controlled productivity signs and duration of the economic use of cows of the Yaroslavl breed and its hybrids with Holstein. For statistical data during determining the strength of influence of factors, we used the procedure generalized linear models (General Linear Models - GLM), and assessment of components of phenotypic variability was performed using multivariate analysis of variance. Our studies have revealed the dependence between controlled signs and the factor «live weight at first calving». When taking into account identified can be we can achieve the increase the duration of the economic use of animals and consequently their lifetime productivity.

Key words: cattle, genetic potential, paratypic factors, the duration of the economic use, lifetime productivity

Introduction

A big part of the phenotypic variability of lifetime productivity of animals due to the influence of paratypic factors. The most significant and true of factors among paratypic factors is «live weight at first calving». The achievement of optimal live weight at first calving (respectively, and at the first insemination) may provide an increase in the duration of the use of animals up to ten days per 1 kg of increasing of body weight (*Konovalov and Malyukova, 2014*). Sexual maturity of heifers is more related to body weight and not to the age that is taken into account in the conditions of intensive cultivation.

The specificity of the modifying effect of the systematic non-inherited factor – «live weight at first calving» with regard to the forces of influence of different technologies on the level and variability of signs of lifetime productivity was studied with the help of such characteristics as the culling of cows, output of the calves and the actual productivity of herds. During the studies similar to ours a big part of scientists came to the conclusion that this factor is complex, it has an impact on the level of the implementation and individual phenotypic variability of signs of lactation, maximize and lifetime productivity. These signs correlate with one another. The two-year interval, the minimum period for assessing the genetic superiority of milk production is considered in the formation of evaluation teams.

Material and methods

Material is prepared on the basis of the «Information database of the Yaroslavl cattle breed» (№ of the state registration is 2013620064), data of the program ARMZS (up to 2009 year) and ARMS -W (№ of the state registration is 2009613920 from 22.07.2009 year), information software module «PAVKA» [3,4]. Farms of the Yaroslavl region with different keeping technology were selected for study: LLC breeding plant «Rodina» (Loose - boxed keeping of cows and equipment of «Westfalia» company), Joint Stock Company, breeding farm named after Dzerzhinsky (captive keeping with the use of installations of «DeLaval» company).

Research objects - pure-bred cows of the Yaroslavl breed and crossbreed cows of the Holstein- Yaroslavl breed having different thorough-bredness of the Holstein breed, in the amount 4776 of them are leavers (1620 in breeding farm named after LLC breeding plant «Rodina», 2772 in breeding farm named after Dzerzhinsky). We determined the realization of the genetic potential estimated on controlling grounds by Kuznetsovsky algorithm, 1983**.** For statistical data in determining the strength of influence of factors procedure we used the procedure of generalized linear models - GLM in the complex «MATLAB 2000» to determine

the strength of influence of factors for statistical processing (*Kuznetsov 2000; Malyukova 2012; Zvereva 2010*).

Results and discussion

If we look at the table 1 and figure 1 we will see that nonlinear dependence is clearly observed when describing the relationship between the duration of the economic use and body weight at first calving. So the correlation coefficient of the sample «the duration x live weight» was -0,13, to the point of optimum (441 ... 450 kg) -0,33, from the optimum point +0,41. Therefore, to achieve optimal body weight at first calving (respectively, at the first insemination) may provide an increase in the duration of the use of animals up to ten days per 1 kg of increasing of body weight. The effect of the interaction was 31,9 *** the power of influence of the factor "live weight at first calving" was 35,5 ***, technology – 23,5 ** unorganized factors – 9,1 *.

Features of the dependence «lifetime productivity x body weight at first calving» are basically close to the estimates "duration x live weight" (identical to the optimum point and the trend of growth). However, they should be considered in the form of multiple correlations (excluding the effect of "duration of use"). Information or calculating multiple correlations is shown in the table 2.

The correlation coefficient between body weight and duration of use excluding the effect of the lifetime productivity was 0,31 **, between body weight and lifetime productivity (with the exclusion of the duration of use) + 0,09 ** between lifelong productivity and duration of use (with the exclusion of living weight) 0,47 ***.

Table 1. Changing of the duration of the economic use of cows and lifetime productivity, depending on body weight at the first calving

Body weight at the first calving, kg	Technology of the keeping cows / Index	Loose - boxed keeping of cows and equipment of «Westfalia»	Captive keeping with the use of installations of «DeLaval» company
361…370	The number of cows	11	6
	The duration of use, days	3008	4105
	The genetic potential of the duration of use, days	2316	2946,9
	The implementation of the genetic potential of the duration of use,%	129,9	139,3
	The lifetime milk yield, kg	14403	19809
	The genetic potential of lifetime milk yield, kg	14403	14927
	The implementation of the genetic potential of lifetime milk yield,%	100,0	132,7
371…380	The number of cows	21	34

	The duration of use, days	3883	2622
	The genetic potential of the duration of use, days	2301	2956
	The implementation of the genetic potential of the duration of use,%	168,8	88,7
	The lifetime milk yield, kg	18774	10791
	The genetic potential of lifetime milk yield, kg	14342	15029
	The implementation of the genetic potential of lifetime milk yield,%	130,9	71,8
381…390	The number of cows	15	64
	The duration of use, days	3837	2662
	The genetic potential of the duration of use, days	2307	2961
	The implementation of the genetic potential of the duration of use,%	166,3	89,9
	The lifetime milk yield, kg	19209	11391
	The genetic potential of lifetime milk yield, kg	14357	15107
	The implementation of the genetic potential of lifetime milk yield,%	133,8	75,4
391…400	The number of cows	20	94
	The duration of use, days	3581	2714
	The genetic potential of the duration of use, days	2306	2966
	The implementation of the genetic potential of the duration of use,%	155,3	91,6
	The lifetime milk yield, kg	20643	11749
	The genetic potential of lifetime milk yield, kg	14326	1516
	The implementation of the genetic potential of lifetime milk yield,%	144,1	77,5
401…410	The number of cows	202	166
	The duration of use, days	3103	3032
	The genetic potential of the duration of use, days	2199	3033
	The implementation of the genetic potential of the duration of use,%	141,1	103,2
	The lifetime milk yield, kg	16282	14396
	The genetic potential of lifetime milk yield, kg	14109	15027
	The implementation of the genetic potential of lifetime milk yield,%	115,4	95,8
411…420	The number of cows	132	155
	The duration of use, days	2591	2667
	The genetic potential of the duration of use, days	2295	2983
	The implementation of the genetic potential of the duration of use,%	112,9	89,4
	The lifetime milk yield, kg	13475	12137
	The genetic potential of lifetime milk yield, kg	14489	15305
	The implementation of the genetic potential of lifetime milk yield,%	93,0	79,3
421…430	The number of cows	46	200
	The duration of use, days	2395	3026
	The genetic potential of the duration of use, days	2319	2935
	The implementation of the genetic potential of the duration of use,%	103,3	103,1
	The lifetime milk yield, kg	15180	14775
	The genetic potential of lifetime milk yield, kg	14375	14970
	The implementation of the genetic potential of lifetime milk yield,%	105,6	98,7
431…440	The number of cows	102	72
	The duration of use, days	2490	3365
	The genetic potential of the duration of use, days	2308	2929
	The implementation of the genetic potential of the duration of use,%	107,9	114,9
	The lifetime milk yield, kg	13945	17846
	The genetic potential of lifetime milk yield, kg	14436	1498

	The implementation of the genetic potential of lifetime milk yield,%	96,6	120,6
441…450	The number of cows	64	174
	The duration of use, days	2182	2699
	The genetic potential of the duration of use, days	2326	2982
	The implementation of the genetic potential of the duration of use,%	93,8	90,5
	The lifetime milk yield, kg	12647	12475
	The genetic potential of lifetime milk yield, kg	14470	15307
	The implementation of the genetic potential of lifetime milk yield,%	87,4	81,5
451…460	The number of cows	75	31
	The duration of use, days	2010	2855
	The genetic potential of the duration of use, days	2337	2949
	The implementation of the genetic potential of the duration of use,%	86,0	96,8
	The lifetime milk yield, kg	12326	14289
	The genetic potential of lifetime milk yield, kg	14501	1492
	The implementation of the genetic potential of lifetime milk yield,%	85,0	95,5
461…470	The number of cows	26	113
	The duration of use, days	2239	2869
	The genetic potential of the duration of use, days	2323	2955
	The implementation of the genetic potential of the duration of use,%	96,4	97,1
	The lifetime milk yield, kg	15553	14335
	The genetic potential of lifetime milk yield, kg	14374	14995
	The implementation of the genetic potential of lifetime milk yield,%	108,2	95,6
471…480	The number of cows	105	46
	The duration of use, days	2187	2854
	The genetic potential of the duration of use, days	2329	2951
	The implementation of the genetic potential of the duration of use,%	93,9	96,7
	The lifetime milk yield, kg	16835	16525
	The genetic potential of lifetime milk yield, kg	14219	14901
	The implementation of the genetic potential of lifetime milk yield,%	118,4	110,9
481…490	The number of cows	176	82
	The duration of use, days	1860	3150
	The genetic potential of the duration of use, days	2382	2936
	The implementation of the genetic potential of the duration of use,%	78,1	107,3
	The lifetime milk yield, kg	14498	17552
	The genetic potential of lifetime milk yield, kg	14383	14787
	The implementation of the genetic potential of lifetime milk yield,%	100,8	118,7
491…500	The number of cows	122	22
	The duration of use, days	1891	3213
	The genetic potential of the duration of use, days	2355	2945
	The implementation of the genetic potential of the duration of use,%	80,3	109,1
	The lifetime milk yield, kg	13065	20494
	The genetic potential of lifetime milk yield, kg	14517	14872
	The implementation of the genetic potential of lifetime milk yield,%	90,0	137,8
501…510	The number of cows	84	44
	The duration of use, days	1800	3323
	The genetic potential of the duration of use, days	2350	2936
	The implementation of the genetic potential of the duration of use,%	76,6	113,2

	The lifetime milk yield, kg	13236	21840
	The genetic potential of lifetime milk yield, kg	14466	14727
	The implementation of the genetic potential of lifetime milk yield,%	91,5	148,3
511…520	The number of cows	63	11
	The duration of use, days	1634	3621
	The genetic potential of the duration of use, days	2348	2943
	The implementation of the genetic potential of the duration of use,%	69,6	123,0
	The lifetime milk yield, kg	11683	29947
	The genetic potential of lifetime milk yield, kg	14513	14869
	The implementation of the genetic potential of lifetime milk yield,%	80,5	201,4
521…530	The number of cows	41	20
	The duration of use, days	1925	3713
	The genetic potential of the duration of use, days	2331	2938
	The implementation of the genetic potential of the duration of use,%	82,6	126,4
	The lifetime milk yield, kg	14545	29223
	The genetic potential of lifetime milk yield, kg	14387	14797
	The implementation of the genetic potential of lifetime milk yield,%	101,1	197,8

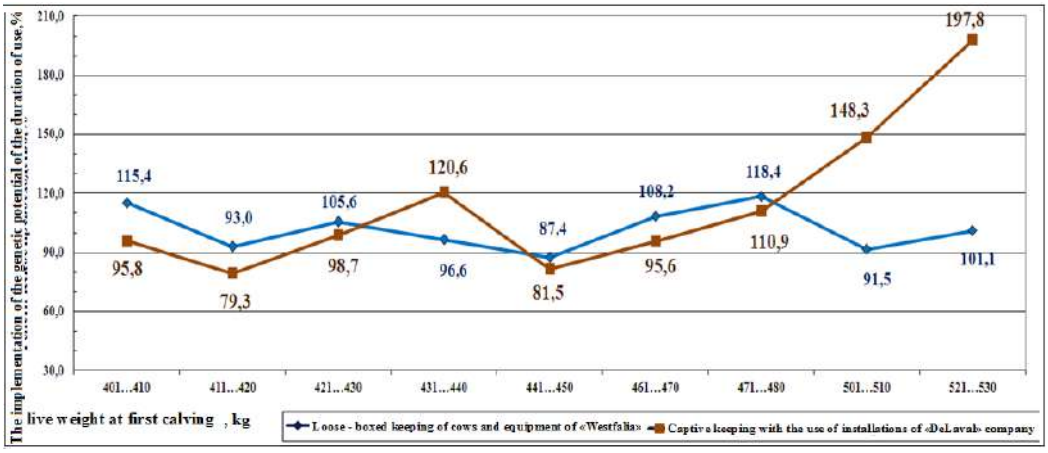

Figure 1. Characteristics of the implementation of the genetic potential of the duration of the economic use in the context of «body weight at first calving»

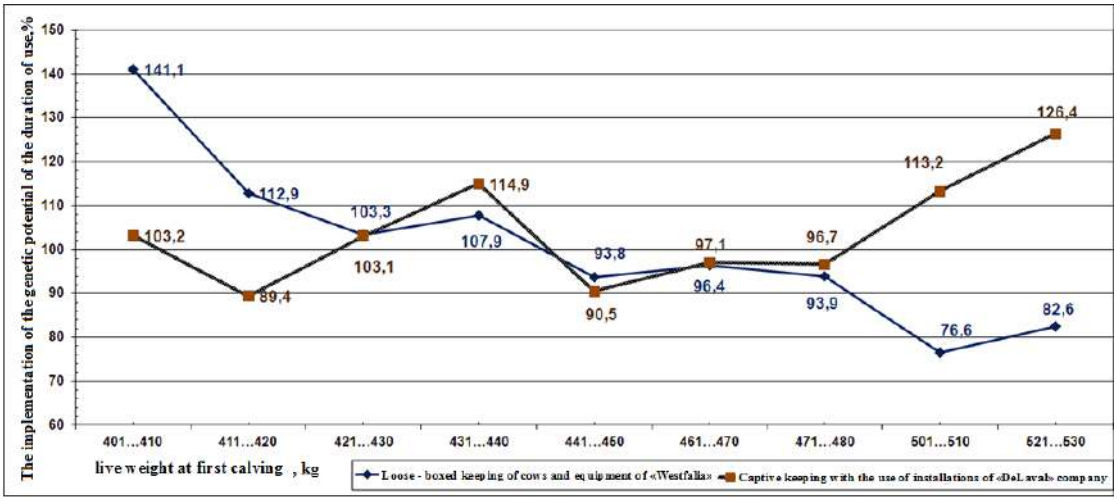

Figure 2. Characteristics of the implementation of the genetic potential in terms of «lifetime yield» in the context of the factor «body weight at first calving»

Table 2. Indicators for the calculation of multiple correlation

Sign	The live weight (x)	The duration of use (y)	The lifetime productivity (z)
The live weight		+0,41	+0,29
The duration of use			+0,53

The results explain the transgressive effect in the range of 530 ... 545 kg of live weight sufficiently. The evaluation of the power of influence showed that interaction effect was 26,8 *** power of influence of factor "live weight at first calving" was 24,4 *** technology – 26,3 ** unorganized factors – 22,5 *.

Conclusion

Nonlinear dependence is revealed between the period of economic use and body weight at first calving, taking into account that the achievement of an optimal body weight at first calving (respectively, and at the first insemination) may provide an increase in the duration of the use of animals up to ten days per 1 kg of the increase in body weight.

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Livestock and fodder production (Yaroslavl, Russia); Fund of Assistance to Small Forms of Enterprises in Science and Technology sphere in program «UMNIK – 2013» (Yaroslavl, Russia).

Uticaj faktora «žive mase pri prvom teljenju» na kontrolisane produktivne osobine i trajanje ekonomsko korišćenje krava jaroslavske rase i njenih meleza sa holštajnom

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Rezime

Jaroslavski region je visoko razvijeni poljoprivredni region Ruske Federacije. Efikasnost vodeće industrije mlečnih goveda u regionu zavisi od nivoa proizvodnje mleka i trajanja ekonomske upotrebe životinja. Ovi indikatori su biološke i ekonomske prirode. Neophodno je da se stvori visoko produktivnih stada i da se poveća trajanje ekonomske upotrebe životinja, a time i doživotnu produktivnost za intenziviranje mlekarstva u regionu. Stoga, cilj našeg istraživanja bio je da se utvrdi uticaj faktora «telesne mase pri prvom teljenju» na produktivne osobine pod kontrolom i trajanje ekonomskog korišćenja krava jaroslavske rase i njenih meleza sa holštajn rasom. U obradi statističkih podataka tokom određivanja snage uticaja faktora, koristili smo postupak opštih linearnih modele (General Linear Models - GLM), a procena komponenti fenotipske varijabilnosti je izvedena korišćenjem multivarijantna analiza varijanse. Naša studija je otkrila zavisnost između osobina pod kontrolom i faktora «žive mase pri prvom teljenju». Kada se uzmu u obzir sve što je identifikovano u okviru ovog ispitivanja, možemo postići povećanje trajanja ekonomske upotrebe životinja i samim tim njihove životne produktivnosti.

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VARIABILITY OF BODY DEVELOPMENT TRAITS IN BUSHA COWS

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Abstract: Busha is small body native breed. It is found where the economic conditions are modest, and agriculture extensive. The intensification of livestock production, and the introduction of more productive, specialized breeds, caused diminished interest in growing Busha. Thus, Busha, as cattle oftriple productive properties, is largely suppressed, so that today we can rarely find examples of the pure breed. To protect specific geno-fund of this breed, the assessment of the level of endangerment of Busha population was carried out, which pointed to the risk status of the population and the necessity of the implementation of conservation programs. The paper analyzes the characteristics of physical development 138 Busha cows in the area of Pirot District, and the influence of non-genetic factors (farm, calving year, calving season) on the variability of traits. The average body weight of Busha cows in the area of Pirot District was 226.07 kg, height at withers 104.33 cm, rump height 104.12 cm,pelvis width 32.52 cm, chest depth 53.97 cm, chest circumference 130.48 cm and body length 119.67 cm. The analysis of the impact of non-genetic factors, showed a highly significant effect ($p < 0.01$) of the farm on all the traits except the width of the pelvis, year of calving exerted a significant effect ($p < 0.01$) on all traits of body development, whereas the calving season exerted no influence ($p > 0.05$) on any of the seven traits that have been studied.

Key words: Busha, body development, fixed factors

Introduction

In Serbia, as in all of Europe, since the 18th century, there was a significant increase in the number of breeds of all farm animals, including the cattle. Over

time, people have invested in knowledge and education, creating new breeds of the combined production traits, usually well adapted to local growing conditions. During the second half of the 20th century, cattle production became intensive and specialized. These changes have caused a change in the definition of the breeding goals. The introduction of selected breeds, suppressed domestic, indigenous breeds of lower genetic potential, which is why there are on the list of endangered or even extinct breeds.

Busha belongs to the group short horn cattle - *Bos brachieceros europeus*. It is grown in an extremely extensive way, and therefore has poorly pronounced production traits, so that the Busha and its crossbreds can be found in the underdeveloped hilly, mountainous and karst areas south of the Sava and Danube rivers.

According to 2009 data released by the FAO and the Institute for Animal Husbandry (2015), number of animals of the indigenous Busha breed in Serbia is 500-1000, and the number of female breeding animals is 350. Institute for Animal Husbandry (2015) also reported that in 2014 there were 365 registered animals of this indigenous breed.

Busha cattle achieve their sexual maturity with 13 - 15 months, and breeding (physical) maturity with 24 (20 - 28) months (*Simijonović 1980, Čobić and Antov, 2002*). Early mating of Busha cows, especially young animals, slows their normal development, primarily due to poor nutrition during pregnancy, the calves remain small and undeveloped due to poor milk yield of the dam. Early mating has very harmful consequences for both mother and calf. Full growth and development of Busha is achieved with 3 - 4 years, and even with 5 years (*Memiši et al., 2003*).

To characterize the morphological characteristics of Busha, previously performed studies are of great importance (*Rako, 1955 Ivanković, 1997*), which provide a good basis for assessing the development of the breed, variability of morphological properties within different populations, and propose a strategy of growing indigenous cattle genome.

Milutinović (1977) states the body dimensions of Metohija strain of Busha cattle -height to withersof 103.97 cm, rump height of 106.88 cm, the chest depth 53.26 cm, chest circumference 140.97 cm and 119.45 cm body length.

Over forty years later *Memiši et al. (2009)*have studied the morphometric characteristics of the population of Busha cows from the area of Šar Mountain with following results: average height at the withers in older cows is 102.3 cm and 127.4 cm body length. The values of these traits in heifers were lower by 7.78 cm (height) and 14.8 cm (body length) compared to the average values in older cows.

Rogić et al. (2011) examined the variability of morphometric characteristics of Busha and Gackocattle in order to preserve indigenous genome.

These authors suggest the following results for the height to withers, chest depth and circumference: in Gackocattle, the following values were measured: 123.67cm, 136.86 cm and 174.22 cm, Busha cattle in the region of the eastern Herzegovina 114.21 cm, 126.71 cm and 167.00 cm, while in western Herzegovina physical measures recorded were 106.11 cm, 117.82 cm and 148.55 cm, respectively. The same authors have found a significant degree of variation in morphological characteristics within the studied populations, and among populations. Identified differences are primarily a result of the effects of different natural conditions and growing conditions as well as the share of alpine cattle genome, with which the Busha was crossbred.

Material and Methods

To test the variability of body development traits, data for 138 cows of Busha breed was used, from 4 farms in the area of Pirot District, in the municipality of Dimitrovgrad. The cows have calved during the period 2005-2013, and all traits were measured immediately after the first calving. The paper investigates the impact of farm, year and season of calving on seven traits of body development: height at the withers, height at the rump, the width of the pelvis, chest circumference, chest depth, body weight and body length. Since Busha cows calf in the spring, only the influence of the first and second season of calving was considered.

Data processing was performed by statistical program *Statistica for Windows version 7* wherein the medium, minimum and maximum values were determined, as well as variability (standard deviation - SD and coefficient of variation - CV). Analysis of the influence of non-genetic factors on the studied traits of body development was performed by the method of Least squares, using a fixed model:

$$Y_{ijkl} = \mu + F_i + G_j + S_k + e_{ijkl}$$

Where:

Y_{ijkl} = Expression of the studied trait in *m* cow, which produced in *i* farm, *j* calving year, *k* calving season

μ = general average of observed trait

F_i = fixed effect of*i*farm

G_j = fixed effect of*j*calving year

S_k = fixed effect of*k*calving season

e_{ijkl} = random error

Results and discussion

Based on the results shown in Table 1, it can be concluded that the average body weight of Busha cows in the area of Pirot District was 226.07 kg, height at withers 104.33 cm, height at rump 104.12 cm width of the pelvis 32.52 cm, the chest depth 53.97 cm, chest circumference 130.48 cm and 119.67 cm body length.

If the results obtained in the present study are compared with the values of other authors, they are lower than the results obtained for Gackocattle and Busha in the eastern and western Herzegovina, which were obtained by *Rogić et al. (2011),* because in this region,the impact of the Tyrolean gray cattle used in crossbreeding with Busha is great.

The height at the withers is greater but the body length is lowercompared toBushacattle in areas ofŠar mountain,recorded by*Memiši et al. (2009).*

Comparing the obtained results with the results for Metohija strain of busha breed arrived at by *Milutinovic (1977)* we see that the results are similar or even lower compared to some properties for this strain of Busha 38 years ago.

Table 1.The mean values and variability of body development traits of Busha cows

Trait	N	Average	Min	Max	SD	CV
Body weight, kg	138	226.07	160.00	275.00	27.04	2.30
Height at withers, cm	138	104.22	98.00	107.00	1.49	0.13
Height at rump, cm	138	104.12	98.00	107.00	1.57	0.13
Pelvis width, cm	138	32.52	30.00	34.00	0.78	0.07
Chest depth, cm	138	53.67	46.00	60.00	2.80	0.24
Chest circumference, cm	138	130.48	125.00	140.00	3.34	0.28
Body length, cm	138	119.67	108.00	131.00	3.39	0.29

The analysis of the impact of non-genetic factors (farm, year and season of calving, Table 2) on the variability of body development traits, highly significant effect ($p < 0.01$) ofthe farm on all traits was found except on the width of the pelvis. The differences found between traits of body development on farms can be explained by the different dietary and housing conditions.

Table 2.The effect of fixed factors on the variability of traits

Trait	F values of studied factors		
	Farm	Year	Season
Body weight, kg	9.16^{**}	14.74^{**}	0.49^{nz}
Height at withers, cm	18.10^{**}	9.30^{**}	2.50^{nz}
Height at rump, cm	27.40^{**}	13.10^{**}	2.10^{nz}
Pelvis width, cm	3.00^{nz}	2.80^{**}	0.40^{nz}
Chest depth, cm	16.06^{**}	13.17^{**}	0.00^{nz}
Chest circumference, cm	10.70^{**}	12.70^{**}	2.90^{nz}
Body length, cm	31.30^{**}	11.20^{**}	6.70^{nz}

$p>0,05^{nz}$, $p<0,05^{*}$, $p<0,01^{**}$

Year of calving was important source of variation that had significant effect ($p <0.01$) on all traits of body development.

Season of calving did not exert influence ($P> 0.05$) on any of the seven properties that were investigated, because cows that have calved in the first season calved in February, so seasonal differences in temperature and humidity, the quality and quantity of available food have not caused significant variation of body development traits.

Conclusion

Based on the results obtained it can be concluded that the average body weight of Busha cows in the area of Pirot District was 226.07 kg, height at withers 104.33 cm, height at rump 104.12 cm, pelvis width 32.52 cm, chest depth 53.97 cm, chest circumference 130.48 cm and 119.67 cm body length.

The analysis of body development traitsshowed that Busha cattle reared in the Pirot District, have small body frame and that according to the morphological characteristics it is most similar to Metohija strain of Busha from 38 years ago, which means that there has been no adequate efforts to improve the traits of body development of this strain.

A high degree of variability ($p <0.01$) of body development traits was establishedin various farms and years of calving, which were primarily the result of different conditions and methods of rearing.

Considering that cows, that have calved in the first season, calved in February, calving season showed no significant effect (p> 0.05) on variation of body development traits.

Busha is small body frame indigenous breed. It is found in the regions with the modest economic conditions, and where the agriculture is extensive. To protect specific geno-fund of the breed, it is necessary to develop and implement the program of its preservation.

Further tests of genomic characteristics of Busha breed, based on molecular markers will show the extent to which the Busha population today is preserved in its original form.

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Varijabilnost osobina telesne razvijenosti krava rase buša

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Rezime

Buša je sitna autohtona rasa. Zastupljena je tamo gde su ekonomske prilike skromne, a poljoprivreda u celini posmatrano ekstenzivna. Inteziviranjem proizvodnje u govedarstvu, I uvođenjem produktivnijih, specijalizovanih rasa, izgubio se interes za gajenje buše. Tako je buša, kao goveče trojnih proizvodnih svojstava, u velikoj meri potisnuta, pa se danas retko mogu naći primerci u čistoj rasi. Radi zaštite specifičnog geno-fonda ove rase izvršena je procena ugroženosti populacije buša, koja je ukazala na rizičan status populacije I neophodnost primene programa očuvanja. U radu su analizirane osobine telesne razvijenosti 138 krava rase buša na području Pirotskog okruga, iuticaj paragenetskih faktora (farma, godina teljenja, sezona teljenja) navarijabilnost osobina. Prosečna telesna masa krava rase buša na području Pirotskog okruga iznosila 226,07 kg, visina grebena 104,33 cm, visina krsta 104,12 cm, širina karlice 32,52 cm, dubina grudi 53,97 cm, obim grudi 130,48 cm, i dužina tela 119,67 cm.Analizom uticaja paragenetskih faktora, utvrđen je visokoznačajan uticaj (p<0,01) farme na sve osobine osim na širinu karlice, godina teljenja ispoljila je značajan uticaj (p<0,01) na sve osobine

telesne razvijenosti dok sezona teljenja nije ispoljila uticaj (p>0,05) ni na jednu od sedam osobina koje su ispitivane.

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HERITABILITY AND REPEATABILITY OF FERTILITY TRAITS OF HOLSTEIN-FRIESIAN BULLS MONITORED IN PROGENY TESTING

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Original scientific paper

Abstract: To test the variability of fertility traits of Holstein-Friesian bulls monitored in progeny test, reproduction data of 342 cows, daughters of 15 bulls were used. Cows have been in production in seven farms of Agricultural Corporation "Belgrade" in the period from 2004 to 2012. The study included the following fertility traits: the average calving age, service period and calving interval. To analyze the influence of genetic and non-genetic sources of variability a mixed model with random effect of bull - sire, the fixed effects of farm, year, season and order of calving was used. The sire is present in variability of all the observed fertility traits ($p < 0.01$), whereas the fixed factors manifested their influence at different levels of statistical significance. The heritability of fertility traits was low: the average calving age – 0.224, service period – 0.087, calving interval – 0.085. Repeatability of mentioned traits was 0.258, 0.111, 0.102, respectively.

Keywords: Holstein-Friesian breed, bulls, fertility traits, heritability, repeatability.

Introduction

Holstein cattle in Europe are grown mainly in the lower regions. In the Republic of Serbia around 100.000 cows and heifers of Holstein - Friesian breed are reared. The most of the animals are grown in Vojvodina and a smaller part in Central Serbia. With the exception of cows that are bred in the vicinity of Belgrade in a very intensive organized production at several large farms (PKB Padinska Skela, PIK Zemun, BD Agro Dobanovci, etc.), number of cattle grown on small farms in Central Serbia is small and amounts to a few thousand (*Lazarević et al., 2013).* The current selection of cattle Holstein - Friesian is mainly focused on improving the

milk traits. Long-term selection of animals for high milk yields has seriously impaired reproductive performance.
Fertility is a feature that is not characterized by yield but is of high economic importance in dairy cattle. Reduced fertility leads to a prolonged calving interval and culling due to reproductive problems (*Jansen, 1985*).

Generally, there is an opinion that a large number of fertility traits is characterized by low heritability (less than 10%), but a large genetic variability. Taking this into account and also the economic importance of fertility traits, certain reproductive traits should be included in the selection criteria (secondary traits) of breeding programs (*Menendez Buxadera and Dempfle, 1997*). According to *Bogdanović et al. (2012)* in a population of Austrian Simmental cattle, relative economic importance of milk production traits, meat production and functional traits is 37:18:45, respectively, while in the population of the Brown cattle breed, relationship between milk production traits and functional traits is 45:55. Reproductive traits that are considered as the functional, such as age at first calving, service period, calving ease and incidence of stillbirth should be emphasized. Breeding for high yields in dairy cattle has led to disruption of fertility traits due to unfavorable genetic correlation between milk yield and fertility (*Pryce et al., 2004).*
In order to improve fertility traits, or to prevent their disruption,it is necessary to put special emphasis on the selection of these traits. Contribution of selection is primarily associated with the estimation of breeding values. Progeny testing, and in particular progeny testing of bulls, and use of the positive bulls, is of great importance. Given that fertility traits are characterized by low heritability, aim of this study was to determine the effect of non-genetic factors and the contribution of bull - sires to the variability of fertility traits.

Material and Methods

To test the variability of fertility traits of Holstein-Friesian bulls monitored in progeny test, reproduction data of 342 cows were used, which were in production on seven farms of Agricultural Corporation "Belgrade" in the period from 2004 to 2012 (Tables 1 and 2).

Table 1. Distribution of calving by year of calving

Calving year	2004	2005	2006	2007	2008	2009	2010	2011	2012	Total
Calving number	7	33	40	138	182	261	211	134	20	1026

Table 2. Distribution of calving by farms

Distribution of calving by farms							Total
1	2	3	4	5	6	7	
390	273	69	129	6	102	57	1026

The cows were daughters of 15 bulls and analysis included the first three calvings (Table 3).

Table 3. Distribution of daughters and calvings by bull-sires

Number	Bull-sire	Number of calvings	Number of daughters
1	1276	60	20
2	1355	111	37
3	1356	93	31
4	1381	36	12
5	1382	63	21
6	1383	30	10
7	1384	123	41
8	1421	51	17
9	1433	75	25
10	1437	36	12
11	1439	54	18
12	1484	105	35
13	1498	117	39
14	1502	33	11
15	1509	39	13
total		1026	342

Within each year the four seasons were observed:

1. Winter: includes cows calved during December, January and February (288 calvings);
2. Spring: includes cows calved during March, April and May (217 calvings);
3. Summer: includes cows calved during June, July and August (272 calvings);
4. Autumn: includes cows calved during September, October and November (249 calvings).

The database contained the following elements: identification number of animals, identification number of the sire, date of birth, dates of the first, second, third and fourth calving and duration of service period. Based on the available data for each cow, age at the first, second and third calving was calculated and then the duration of calving interval. The analysis included three repetitions for each trait.

The study included the following fertility traits:

- Average age at calving (days);
- Service period (days);
- Calving interval (days).

Statistical analysis of data obtained during the progeny testing of Holstein-Friesian bulls was divided into three parts.

The first part of the analysis included the determination of the basic variation-statistical parameters. Descriptive statistics analysis was performed using the statistical program *StatSoft.Inc (2004), Statistica for Windows version 7.*

The second part of the data processing included the identification of the different impacts on the variability of traits in progeny test. The variability of fertility traits was assessed by the method of least squares *LSMLMW*. To analyze the influence of genetic and non-genetic sources of variability a mixed model was used with random effect of bull-sire, fixed effects of farm, year, season and the order of calving.

Mixed model for analyzing the impact of genetic and non-genetic sources of variability on fertility traits in progeny test:

$Y_{ijklmn} = \mu + O_i + F_j + G_k + S_l + R_m + e_{ijklmn}$
where:

- Y_{ijklmn}: studied trait,
- μ: population average for observed trait,
- O_i: random effect of i-th sire (i=1,…,15),
- F_j: fixed effect of j-th farm (j=1,…,7),
- G_k: fixed effect of k-th calving year (k=1,…,9),
- S_l: fixed effect of l-th calving season (l=1,2,3,4),
- R_m: fixed effect of m-th order of calving (m=1,2,3),
- e_{ijklmn}: random error with N characteristics$(0,\sigma^2)$.

In the third part of the analysis, components of variance, heritability, repeatability and correlation of fertility traits were calculated. Components of variance were

calculated by the method REML (Restricted Maximum Likelihood) within VARCOMP procedures (SAS 9.3, 2012).
For the calculation of heritability (h^2) and repeatability (r) coefficients, the following formulas were used:

$$h^2 = \frac{\delta^2_a}{\delta^2 p} \qquad r = \frac{\delta^2 a + \delta^2 pe}{\delta^2 p}$$

where:
$\delta^2 a$ - additive genetic variance;
$\delta^2 pe$ - variance of permanent/fixed environmental factors;
$\delta^2 p$ - Total (phenotypic) variance, consisting of additive variance, dominance variance, variance of interactions (epistasis), the variance of permanent/fixed effect of environmental factors and the error variance
($\delta^2 p = \delta^2 a + \delta^2 d + \delta^2 i + \delta^2 ep + \delta^2 pe + \delta^2 e$).

Results and Discussion

Table 4 presents the descriptive statistical indicators and variability of fertility traits monitored in progeny test of the Holstein-Friesian bulls. Average duration of calving interval was 411.69 days with a coefficient of variation of 18.78%. *M'hamdi et al. (2010)* report a slightly higher duration of calving interval (444.2), while *Ghiasi et al. (2011)* in their research obtain lower values 393.85 and similar results are reported by *Hoekstra et al. (1994).* Minimum calving interval of 237 days indicates early/premature partus or pregnancy ending in abortion, because the normal duration of gestation in cows of the Holstein – Friesian breedas reported in the research of *Petrović (1993),* is 275.37 days. Maximum duration of calving interval is 758 days, which is, from the economic and technological aspect, extremely unfavorable because one calving is lost and lactation postponed.
The duration of the calving interval is directly affected by the duration of service period. Average service period is 134.51 days and is characterized by high variability (57.33%). *Petrović (1993)* states a shorter service period, in first calving heifers 105.9 days whereas in cows service period lasted 119.6 days. *M'hamdi et al. (2010)* in their investigation state the average service period of Holstein - Friesian cows in Tunisia of 150.9 days.

Table 4. Mean values and variability of fertility traits in progeny test of Holstein-Friesian bulls

Trait	N	μ	Min	Max	Sd	Cv (%)
Calving interval, days	1026	411.69	237	758	77.32	18.78
Service period, days	1026	134.51	29	474	77.12	57.33
Average calving age, days	1026	1182.51	634	2008	350.30	29.62
Average age at first calving, days	342	770.34	634	1175	50.66	6.58
Average age at second calving, days	342	1182.84	1024	1571	92.55	7.82
Average age at third calving, days	342	1594.34	1347	2008	131.27	8.23

The maximum duration of service period is 474 days. There are a number of reasons for this prolonged service period: long-term selection for high milk yield, poor farm management, poor conception, silent estrus and malnutrition.
Age at first insemination is one of the significant traits from a biological and economic point of view. Premature bred heifers are lagging behind in growth, giving small and undeveloped calves and their milk yield deteriorates. However, intensive rearing in the period of growth enables early breeding of heifers. The two main criteria for the breeding time are: age, or the age and development of heifers, which are considered to be physically developed for fertilization when they reach ⅔ to ¾ of body weight of cows *(Petrović, 1993).*

The cows calved for the first time at the age of 770.34 days, the same results are stated by *Petrović (1993).* The average age at the first, second and third calving is characterized by less variability than other fertility traits, with the variability increasing from the first to the third calving.
Bull-sire is present in variability of all observed fertility traits ($p < 0.01$), while the farm at a high level ($p < 0.01$) exerted influence only on calving interval and duration of service period (Table 5). The effect of season was present in the variability of the calving interval and service period, without significant effect on the average age at calving. Seasonal variations in temperature and humidity, the quality and quantity of food available caused a significant variation of reproductive performance. Order of calving did not influence the variation of calving interval and service period ($p > 0.05$), while the statistically significant effect ($p < 0.01$) was recorded on the average age at calving. *M'hamdi et al. (2010)* report a statistically significant effect ($p < 0.01$) of farm, year and order of calving on calving interval

and duration of service period. The effect of the year is present in variability of all observed reproductive traits but on a different level of statistical significance.

Table 5: Influence of genetic and non-genetic factors on the variability of fertility traits in progeny testing of Holstein-Friesian bulls, F value

Trait	F values of studied effects				
	Bull-sire	Farm	Year	Season	Order of calving
	df_1=14	df_1=6	df_1=8	df_1=3	df_1=2
	df_2=992	df_2=992	df_2=992	df_2=992	df_2=992
Calving interval, days	4,69**	2,41**	1,87*	5,08**	$0,94^{nz}$
Service period, days	4,67**	2,41**	1,75*	5,25**	$1,24^{nz}$
Average calving age, days	9,64**	$1,58^{nz}$	4,64**	$1,12^{nz}$	2580,39**

$p>0,05^{nz}$, $p<0,05^{*}$, $p<0,01^{**}$

Table 6 shows the components of variance, heritability and repeatability of reproductive traits.

According to the literature data, heritability of fertility traits is low, below 0.1 (*Ghiasi et al., 2011).* The obtained heritability of fertility traits ranged from 0.085 (calving interval) to 0.224 (average age at calving). Slightly lower values are reported by *M'hamdi et al. (2010). Ghiasi et al. (2011)* in the population of Iranian Holstein received slightly lower values of heritability, 0.074 for calving interval and 0.076 for service period. *Hoekstra et al. (1994)* report heritability of 0.03 for calving interval.

Repeatability of fertility traits is characterized by somewhat higher values than heritability coefficients, Table 6. Repeatability coefficient is used to calculate the potential production of the animal on the basis of the first production results (*Stanojević et al, 2013*). Repeatability ranged from 0.102 (calving interval) to 0.258 (average age at calving). *M'hamdi et al. (2010)* report slightly higher values: 0.152 for calving interval and 0.135 service period.

Table 6. Components of variance, heritability and repeatability of fertility traits monitored in progeny testing of Holstein-Friesian bulls

Trait	σ_a	σ_{pe}	σ_p	h^2	r
Calving interval	511,750	99,750	5987,917	0,085	0,102
Service period	524,865	146,306	6034,996	0,087	0,111
Average calving age	2423,000	369,700	10804,941	0,224	0,258

There is a strong genetic correlation between fertility traits, Table 7. There is a complete genetic correlation between calving interval and service period, 0.999. *Ghiasi et al. (2011)* report the same values of genetic correlations. This is a strong

association between fertility traits originating from the fact that certain traits are calculated directly from the others.

Table 7. Genetic correlations of fertility traits

Trait	Calving interval	Service period	Average calving age
Calving interval	1,000	0,999	0,908
Service period	–	1,000	0,895
Average calving age	–	–	1,000

In order to improve, or at least stop the negative trend of fertility traits, it is necessary to place special emphasis on these traits in the selection. Finally, there are great expectations in the use of genomic selection in order to improve reproductive traits *(M'hamdi et al., 2010).*

Conclusion

Based on the results of the analysis of fertility traits of Holstein-Friesian bulls monitored in progeny testing, the following can be concluded: the average duration of calving interval was 411.69 days with a coefficient of variation of 18.78%, while the average duration of service period was 134.51 days and characterized by high variability (57.33%). The cows calved for the first time at the age of 770.34 days. Bull-sire is present in variability of all observed of fertility traits ($p < 0.01$), while the fixed factors (farm, year, season and order of calving) showed their influence at the different level of statistical significance. The heritability of fertility traits was low: the average age at calving, 0.224, service period 0.087 and calving interval 0.085. Repeatability of mentioned traits was 0.258, 0.111 and 0.102, respectively. There was a strong positive correlation between the studied fertility traits.

Looking at the obtained results it is evident that it takes a lot of effort in the selection of fertility traits in herds of dairy cattle. Given that fertility traits are characterized by low heritability and high genetic variability, and significant share in the profitability of milk production, certain reproductive traits should be included as the selection criteria in breeding programs.

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Heritabilitet i repitabilitet osobina plodnosti bikova holštajn frizijske rase praćenih u progenom testu

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Rezime

Za ispitivanje varijabilnosti osobina plodnosti holštajn-frizijskih bikova praćenih u progenom testu iskorišćeni su podaci o reprodukciji 342 krave, kćeri 15 bikova. Krave su proizvodile na sedam farmi Poljoprivredne Korporacije "Beograd" u periodu od 2004 do 2012 godine. Istraživanje je obuhvatilo sledeće osobine plodnosti: prosečan uzrast pri teljenju, trajanje servis perioda i međutelidbeng intervala. Za analizu uticaja genetskih i negenetskih izvora varijabilnosti upotrebljen je mešoviti model sa slučajnim uticajem bika - oca, fiksnim uticajem farme, godine, sezone i rednog broja teljenja. Bik – otac prisutan je u varijabilnosti svih posmatranih osobina plodnosti ($p<0,01$) dok su fiksni faktori svoj uticaj ispoljili na različitom nivou statističke značajnosti. Heritabilitet osobina plodnosti je nizak: prosečan uzrast pri teljenju, 0,224, trajanje servis perioda 0,087, međutelidbeni interval 0,085. Repitabilitet navedenih osobina je 0,258, 0,111, 0,102, odgovarajuće.

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IMPACT OF RELEVANT ENVIRONMENTAL FACTORS ON THE PHENOTYPIC VARIABILITY OF MILK PRODUCTION TRAITS OF BLACK-AND-WHITE DAIRY CATTLE IN THE FIRST THREE LACTATIONS

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Abstract: The aim of this study was to analyse the impact of relevant environmental factors on genetically improved Black-and-White dairy cows in the period from 1993 to 2010. The investigation of the phenotypic variability of major milk production traits showed that systematic non-genetic factors, such as farm, year, season of calving and sequence of lactation, have a highly significant impact on the manifestation of the observed traits ($p<0.01$). The difference between the maximum and minimum average milk production traits per farm was 824.96 kg, or about 12% compared to the overall average, due to different management on farms. A negative phenotypic trend for milk production in the observed population was present from 1993 to 2002, as a result of generally unfavourable situation in the country during the nineties. The cows that calved in autumn achieved higher production than those that calved during summer. The largest positive deviation from the overall average milk yield was recorded in the third standard lactation (+359.21 kg), whereas the largest negative deviation was recorded after the first standard lactation (-580.82 kg). This test confirmed that models for estimating breeding values must include the impacts of farm, year and season of calving. Those factors can be seen to have a unique impact, when their interactions are taken into account, which makes the parameters more reliable.

Key words: milk production traits, phenotypic variability, environmental factors, Black-and-White cattle breed.

Introduction

Black-and-White and Holstein-Friesian dairy cattle are characterised by vast potential for high milk yields. Relevant milk production traits - milk yield, fat yield and protein yield are properties that are subject to continuous phenotypic variation. Besides heredity, the manifestation of these traits is also affected by many factors of the environment. The most common studied are the impacts of farm, year of production, calving season, the sequence of lactation, and age at calving, as well as other relevant non-genetic factors that can affect the overall variability of milk production traits (*Stojić et al., 2013; Đedović et al., 2013, 2012, 2003; Stanojević et al., 2013, 2012; Petrović et al., 2009; Živanović, 2003;Trifunović et al., 2002*).
The impact of farm/herd involves a number of factors that affect the phenotypic manifestation of milk production traits (housing conditions and care, diet, age and health status of the herd, applied management) and it usually has the largest share in the variation of these traits (*Stojić, 1996*). A year, as a period in which cattle produce milk, has its own characteristics that can be seen through the activity of climatic factors, methods of preparation and use of feed, applied farming practices, and general economic conditions that may be reflected in the actual production of milk (*Beskorovajni, 2014*).
It is known that high temperatures and humidity can adversely affect not only yields, but also the reproductive and health status of cattle (*Beskorovajni et al., 2012*). Cows calved in summer usually have lower production results than cows calved in autumn and winter (*Trivunović, 2006*).
Environmental factors can often be the limiting factor in assessing genetic variation, so they should be included in the models for estimating breeding values (*Popovic, 2015*). In doing so, the factors of farm, year and season can be seen to have a unique impact. Hence their interactions are taken into account, which makes the parameters more reliable.

Materials and Methods

The phenotypic variability of milk production traits were established on the basis of production data on 6022 cows, daughters of 62 Black-and-White and Holstein-Friesian bulls. The cows were raised on seven farms of the Agricultural Corporation "Belgrade" in the period from 1993 to 2010. To test the variability of milk production traits the authors used production indicators from the first three standard lactations: milk yield (MY-305), fat content (FP-305), fat yield (FY-305) and the yield of 4% FCM-305. The analysis included a total of 18066 lactations.

The correction of milk yield for 4% fat was performed by Gaines - Davidson's formula:

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

where: M - milk yield
F - milk fat yield

Months of cows' calving were divided into four groups/seasons: 1 - January, February, March; 2- April, May and June; 3 - July, August, September; 4 - October, November, December.
For the purpose of analysing the systematic impacts of farm, year, season of calving and sequence of lactation the authors used the method of least squares, according to the LSMLMW program (*Harvey, 1990*). The average values and phenotypic variability of milk production traits were calculated by using the same program and the following model:

$$Y_{ijklm} = \mu + F_j + G_k + S_l + L_m + e_{ijkl}$$

Where:
Y_{ijklm} - phenotypic manifestation of the test features
μ - general population's average
F_i - fixed effect of the j-farm (j = 1, ... 7)
G_k - fixed effect of k-year of calving (k = 1, ... 18)
S_l- impact of fixed l-calving season (l = 1, ... 4)
L_m - fixed effect of lactation (m = 1, 2, 3)
e_{ijkl} - random error

Results and Discussion

By applying the mixed model that involves farm, year, season of calving and sequence of lactation, the authors obtained F-value (Table 1). The observed systematic factors had a high impact on the phenotypic manifestation of milk production traits in the first three standard lactations ($p<0.01$). Determination coefficients were relatively high, which confirms the accuracy of the applied model.

Table 1. Impact of systematic factors on milk production traits in standard lactation

Effects	d.f.$_1$	F - value			
		MY-305	FP-305	FY-305	4%FCM-305
Farm	6	121.431**	312.009**	199.564**	159.037**
Year	17	131.991**	96.813**	111.895**	121.873**
Season	3	155.297**	19.577**	131.310**	147.396**
Lactation	2	651.388**	18.432**	540.138**	614.004**
R^2		0.445	0.255	0.390	0.421

** p < 0.01 * p < 0.05 ^{NS} p > 0.05

Different management on farms (applied farming practices, nutrition, care, hygiene, health care, the process of work organisation and management) resulted in uneven milk yields in standard lactation. The difference between the maximum and minimum average milk yield per farm was 824.96 kg, or about 12% compared to the overall average.

Table 2. Deviation (q) and deviation error (Sq) from overall average (μ) milk production traits in standard lactation, observed by farms

Farm	n	MY-305, kg		FP-305, %		FY-305, kg		4% FCM-305, kg	
		q	Sq	q	Sq	q	Sq	q	Sq
μ	*18066*	*7056.44*	*28.07*	*3.61*	*0.005*	*252.83*	*1.01*	*6615.00*	*25.83*
1	2904	381.97	23.93	0.13	0.005	25.29	0.80	532.17	22.03
2	2988	324.22	23.86	-0.12	0.005	2.15	0.86	161.92	21.96
3	2301	-104.74	26.24	0.07	0.005	-7.22	0.95	-52.74	24.15
4	2409	-31.51	25.57	-0.04	0.005	-4.93	0.92	-86.54	23.53
5	2673	-14.80	24.52	-0.08	0.005	-6.59	0.89	-104.80	22.57
6	3057	-442.99	23.43	-0.02	0.005	-17.06	0.85	-433.08	21.57
7	1734	-112.14	29.22	0.07	0.006	1.86	1.06	-16.93	26.89

Farm 6 had most lactations, but the lowest average milk yield and fat yield. The number of cows that produced milk was lowest on farm 7 (Table 2). The cows on farm 1 demonstrated superiority over their peers. On average, they produced 381.97 kg of milk, 25.29 kg of milk fat and 532.17 kg of 4% FCM more than the overall average. The lowest results were obtained on farm 6, where the average deviation in milk yield, fat yield and 4% FCM was - 442.99 kg,- 6.17 kg and - 433.08 kg, respectively.

Each year had its own specificities, manifested through climatic factors, methods of preparation and use of feed, the organisation on farms, and general situation in

agriculture that affected the entire business, so the phenotypic manifestation of milk production traits oscillate during the test period, as well.

Table 3. Deviation (q) and deviation error (Sq) from overall average (μ) milk production traits in standard lactation, observed by years of calving

Year	n	MY-305, kg		FP-305, %		FY-305, kg		4% FCM-305, kg	
		q	Sq	q	Sq	q	Sq	q	Sq
μ	*18066*	*7056.44*	*28.07*	*3.61*	*0.005*	*252.83*	*1.01*	*6615.00*	*25.83*
1993	18	-1026.05	295.92	0.10	0.058	-31.02	10.69	-875.77	272.34
1994	134	-88.16	122.43	0.05	0.024	-0.05	4.42	-36.04	112.68
1995	129	-562.70	121.67	0.02	0.024	-18.46	4.40	-501.89	111.98
1996	256	-825.29	86.55	0.05	0.017	-26.08	3.13	-721.31	79.66
1997	268	-910.76	84.07	0.11	0.016	-25.01	3.04	-739.42	77.37
1998	322	-738.06	77.14	0.10	0.015	-18.80	2.79	-577.25	70.99
1999	653	-1123.51	58.43	0.04	0.038	-37.11	2.11	-1006.15	53.77
2000	970	-1590.60	51.66	0.05	0.010	-53.76	1.87	-1442.57	47.54
2001	1483	-732.73	43.30	0.08	0.008	-20.02	1.56	-593.41	39.85
2002	1797	10.01	39.34	0.10	0.008	8.58	1.42	132.77	36.20
2003	1723	3.03	40.22	0.03	0.008	2.89	1.45	44.61	37.04
2004	1779	278.41	41.40	-0.09	0.008	3.93	1.50	170.35	38.10
2005	1731	697.38	43.86	-0.15	0.009	14.36	1.58	494.35	40.36
2006	2173	893.74	43.04	-0.23	0.008	14.50	1.55	574.96	39.61
2007	2012	1110.46	45.31	-0.17	0.009	26.35	1.64	839.40	41.70
2008	1637	1429.46	50.43	-0.98	0.010	43.60	1.82	1225.79	46.41
2009	875	1590.10	60.82	-0.04	0.011	53.70	2.20	1441.62	55.97
2010	106	1585.26	131.18	0.05	0.026	62.39	4.74	1569.96	120.72

The largest positive deviation from the overall average milk yield was recorded in 2009 (+1590.10 kg), and the largest negative deviation in 2000 (- 1590.60 kg). As for the other properties, the largest positive deviation was recorded in 2010 (+62.39 kg in fat yield and +1569.69 kg in 4% FCM), while the largest negative deviation was recorded in 2000 (- 53.76 kg in fat yield and - 1442.57 kg in 4% FCM). As for fat content, the largest positive deviation (+ 0.11%) was recorded in 1997, and the largest negative deviation (- 0.23%) in 2006 (Table 3).

Differences in yields of milk, fat, 4% FCM and fat content by years of calving were statistically highly significant, which in line with those obtained by *Katok and Yanar (2012).*

Table 4 presents the indicators of milk production traits per calving season. Most of the milk was produced by cows calved in the fourth season (autumn), while the lowest milk yield was achieved by cows calved in the second season (summer).

Table 4. Deviation (q) and error deviation (Sq) from overall average (μ) milk production traits in standard lactation, observed by calving season

Season	n	MY-305, kg		FP-305, %		FY-305, kg		4% FCM-305, kg	
		q	Sq	q	Sq	q	Sq	q	Sq
μ	*18066*	*7056.44*	*28.07*	*3.61*	*0.005*	*252.83*	*1.01*	*6615.00*	*25.83*
1	4374	-7.17	17.63	-0.02	0.003	-1.44	0.64	-24.53	16.22
2	4308	-231.51	17.44	0.01	0.003	-7.94	0.63	-211.70	16.05
3	5061	-111.98	16.58	0.02	0.003	-2.44	0.60	-81.45	15.26
4	4323	350.65	17.48	-0.01	0.003	11.83	0.63	317.68	16.09

When compared to the overall average, the cows calved from October to December achieved higher milk yields (+350.65.50 kg). As for the other properties, deviations were as follows: + 11.83 kg (fat yield) and + 317.68 kg (4% FCM). The largest negative deviations from the average indicators in the standard lactation were observed in cows calved from April to June: - 231.51 kg in milk yield, - 7.94 kg in fat yield, - 211.70 kg in 4% FCM. These values are in line with studies on similar populations of the genetically improved Black-and-White cattle (*Beskorovajni, 2014, 1999; Lateef et al., 2008*).

This effect was mostly manifested through the diet and microclimate in buildings. A negative impact of high temperatures and humidity cease during the first months of autumn, so animals are no longer under these stress factors and can therefore use nutrients more efficiently, thus increasing milk production.

The overall average and deviation from the overall average milk production traits in the standard lactation, analysed by the sequence of lactation, are shown in Table 5.

Table 5. Deviation (q) and error deviation (Sq) from overall average (μ) milk production traits, observed by sequence of lactation

Lact.	n	MY, kg		FP, %		FY, kg		4% FCM, kg	
		q	Sq	q	Sq	q	Sq	q	Sq
μ	*18066*	*7056.44*	*28.07*	*3.61*	*0.005*	*252.83*	*1.01*	*252.83*	*1.01*
1	6022	- 580.82	16.09	0.01	0.003	-19.11	0.58	-518.97	14.81
2	6022	221.60	14.24	-0.01	0.002	7.43	0.51	200.15	13.11
3	6022	359.21	16.17	-0.01	0.003	11.68	0.58	318.82	14.88

The highest yields were achieved in the third lactation, which is consistent with previous studies (*Bunevska et al., 2013; Trifunovic et al., 2002*). As the average milk yield increases from the first to subsequent lactation, it is

necessary to include this factor in models for estimating breeding values (*Trivunović, 2006*)
The largest positive deviation from overall average milk yield was in the third standard lactation (+359.21 kg), whereas the largest negative deviation was - 580.82 kg in the first standard lactation. The cows produced milk with the highest fat content in the first lactation, by + 0.01% more than the average.

Conclusion

Non-genetic factors (farm, year, season of calving and sequence of lactation) had a significant impact on the manifestation of the observed milk production traits during the first three standard lactations ($p < 0.01$).
Although farming practices on were quite similar, the difference of 824.69 kg in the production recorded on the best-ranking and worst-ranking farms proved to be statistically significant, when compared to the overall average.
A negative phenotypic trend of milk production in the observed population was present for almost a decade (from 1993 to 2002), as a result of deteriorating production conditions in the nineties.
Bearing in mind the importance of environmental factors on the variability of milk production traits, it is essential to continuously improve raising conditions along with improving the genetic basis of cattle. It is better to include as many non-genetic factors as possible in the models of estimating breeding values, so that the obtained parameters would be more reliable. The coefficients of determination (R^2) in this test had relatively high values, thus confirming the accuracy of the model used.

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Uticaj važnijih faktora okoline na fenotipsku varijabilnost osobina mlečnosti krava oplemenjene crno bele rase u prve tri standardne laktacije

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Rezime

Cilj ovog istraživanja bio je analiza uticaja važnijih faktora okoline u kojoj su proizvodile krave oplemenjene crno bele rase u periodu od 1993. do 2010. godine. Ispitivanje fenotipske varijabilnosti važnijih osobina mlečnosti pokazalo je da delovanje sistematskih negenetskih faktora - farme, godine, sezona teljenja i laktacije po redu ima visoko signifikantan uticaj na ispoljenost posmatranih svojstava ($P< 0.01$). Razlika između maksimalne i minimalne prosečne mlečnosti po farmama iznosila je 824.96 kg ili oko 12% u odnosu na opšti prosek, što je posledica različitog menadžmenta na farmama. Negativan fenotipski trend proizvodnje mleka u posmatranoj populaciji bio je prisutan od 1993 do 2002. godine, kao posledica opštih nepovoljnih prilika u zemlji tokom devedesetih godina. Krave koje su se telile u jesenjim mesecima postigle su veću proizvodnju nego one koje su se telile tokom letnje sezone. Najveće pozitivno odstupanje od opšteg proseka za prinos mleka bilo je u trećoj standardnoj laktaciji (+359.21 kg), a najveće negativno kod krava sa završenom prvom standardnom laktacijom (-580.82 kg). Ovo ispitivanje je potvrdilo da je u modele za procenu priplodne vrednosti neophodno uključiti uticaje farme, godine i sezone teljenja. Oni se mogu posmatrati i kao jedinstven uticaj, čime se obuhvataju njihove međusobne interakcije, što doprinosi pouzdanosti dobijenih parametara.

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ESTIMATION OF VARIANCE COMPONENTS AND HERITABILITY FOR MILK TRAITS OF SIMMENTAL COWS IN VOJVODINA

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Abstract: Purebred Simmental is excellent dual purpose cattle breed. Production traits are revenue traits in the breeding of dairy cattle; milk yield is one of the most economically important traits in dairy industry, so, all modern dairy cattle breeding programs include production traits. There is considerable phenotypic variability of production traits of dairy cattle in Vojvodina. The aim of this study was to estimate the variance components and heritability for milk traits (milk, fat and protein yield, fat and protein content) of Simmental cows in Vojvodina. Data relating to 4736 purebred Simmental cows and were obtained from the Main breeding organization (Faculty of Agriculture in Novi Sad, Department of Animal Science). Descriptive statistical parameters were calculated. The fixed effect and their significant levels were calculated by general linear model (GLM) in Statistica 12 (StatSoft, 2015). Estimation of variance components and heritability were conducted by restricted maximum likelihood (REML). Average milk yield was 5760 kg, content and yield of milk fat was 3,88% and 224 kg, and the content and yield of protein was 3,28% and 188,8 kg. Breeding region had high significant effects on all observed milk traits. Calving year and season did not have significant effect only on fat content, while lactation number did not have significant effect only on protein content. Heritability for milk traits was ranged from 0,32 (fat content) to 0,63 (fat yield). Heritability values shows that the additive genetic variance has a significant proportion in considerable phenotypic variability of production traits of Simmental cows in Vojvodina, which is why it is justified to expect significant improvement of production traits of Simmental cows in Vojvodina through genetic selection

Key words*: Simmental cows, milk production traits, variance components, heritability*

Introduction

The dairy production is a complex system that requires optimal management of all its components to achieve sustainability. The dairy cattle are the main component of the system, and virtually all biological or physiological processes, therefore, the performance of dairy cattle are to a lesser or greater extent under genetic control. Purebred Simmental is excellent dual purpose cattle breed. Milk production is a very complex system that requires optimal management practices and the compliance of all activities on dairy farms. Production traits (milk, fat and protein yield, fat and protein content) are revenue traits in the breeding of dairy cattle; milk yield is one of the most economically important traits in dairy industry (*Hu et al., 2010*). Overall production, especially the yield of milk fat and protein, remain a significant factor in the selection and when making culling decisions. Selective breeding of dairy cattle combined with artificial insemination, has led to a significant improvement of dairy cattle, especially through enormous improvement of production traits of dairy cows. But, such intensive selection has had effects on other performance of modern dairy cows; one of the most significant issues in dairy production in recent times is a falling rate of cow fertility (*Williamson, 2014*). In this sense, the modern breeding programs for dairy cattle should be more balanced. Although modern dairy cattle breeding programs and goals include more and more functional (*Pfeiffer et al., 2015*) and conformation traits (over and above reflective of health, reproductive performance and longevity) (*Berry, 2014*), they all include production traits as most important component, especially for Simmental.

Milk production traits are determined by genetic and non-genetic factors. There is considerable phenotypic variability of dairy cattle for production traits. The heritability is the ratio of additive genetic variance to the total phenotypic variance. Variance components and genetic parameters are needed for genetic improvement programs to predict the breeding values of candidates for genetic selection, to choose among mating plans and to predict selection response (*Montaldo et al., 2012*). Consequently we need partition the variance into its basic genetic and non-genetic components, i.e., into its additive and residual-environmental components. Thus, the evaluation of genetic parameters, heritability in particular, is crucial for better understanding of genetic mechanism and designing of effective programs for genetic improvement of dairy cattle. Genetic improving of production traits primarily depend on efficient exploitation of additive genetic variance. This requires sophisticated system of milk recording and analysis, increasing the share of cows included in milk recording program and accurate estimates of genetic parameters for the traits of interest.

The objective of this study was to estimate the variance components and heritability for milk traits of Simmental cows of all parities in Vojvodina by using the data of the official milk-recording program. The test-day model has become a model of choice for national genetic evaluation of production traits in many countries (*International Bull Evaluation Service, 2009*). Although the main breeding organization for Simmental in Vojvodina is able to deal with test day records in this study we have used a 305-day animal model. The program included individual milk measurements collected using AT4 methodology (*ICAR, 2002*) with monthly visits and milking controls (interchangeably, one month morning, second afternoon).

Material and method

Data relating to 4736 purebred Simmental cows and were obtained from the Main breeding organization (Faculty of Agriculture in Novi Sad, Department of Animal Science). The calving records were gathered from 8 lactations, during 2013-2014. Milk records were collected using AT4 methodology (*ICAR, 2002*), once monthly while records were combined from morning and afternoon milking interchangeably.

For calculation of average values and variability of milk traits (milk, fat, protein yield and fat and protein content) descriptive statistics was performed. For this purpose, standard statistical parameters were calculated: mean, standard error of the mean, minimum, maximum, standard deviation and coefficient of variation.

In order to analyze and determine the fixed effect and their significant levels we applied general linear model (GLM) in Statistica 12 (*StatSoft, 2015*) with the following model:

$$Y_{ijklm} = \mu + R_i + Yc_j + Sc_k + L_l + e_{ijklm}$$

Where:
Y is milk, fat and protein yield (kg), fat and protein content; μ is general average; R is fixed effect of breeding region; Yc is fixed effect of calving year; Sc is fixed effect of calving season; L is fixed effect of lactation number; e is random residual with assumed normal distribution $N(0,\boldsymbol{\sigma}^2)$

Genetic analyses (estimates component of variance and heritability) were conducted with single trait animal model and restricted maximum likelihood (REML) procedures using the software WOMBAT (Meyer, 2007). The basic model was fitted as follows:

$$y = Xb + Za + e$$

Where is:
y – vector of observations;
X and Z – the incidence matrices relating observation to effect;
b – a vector of fixed effects;
a – a vector of additive genetic effect;
e – a vector of unknown residual effects.

Results and discussion

Descriptive statistical parameters of analyzed milk traits and the results of the analysis of variance are presented together with standard deviations and minimum-maximum values in Table 1.

Table 1.Mean and variability of milk traits in standard lactation (305 days)

Traits	N	$\bar{x}$	$S_{\bar{x}}$	Min	Max	SD	CV
Milk yield, kg	4736	5760	16,95	2422	13213	1167,10	20,26
Fat yield, kg	4472	224,0	0,76	67,0	561,0	51,49	22,98
Protein yield, kg	4645	188,8	0,58	72,0	418,0	40,04	21,21
Fat, %	4472	3,88	0,01	2,00	5,02	0,44	11,44
Protein, %	4645	3,28	0,003	2,20	4,69	0,26	7,94

The average milk yield is higher in relation to Croatian Simmental (5030 kg) *(CAA, 2015)*) as well as in relation to Slovenian Simmental (5283 kg) *(Kmetijski Institut Slovenije, 2014.)*), but the average content of milk fat and protein were lower in relation to Croatia (fat 4,01%, protein 3,35%) and Slovenia (fat 4,05%, protein 3,37%). The average milk yield and milk fat content results are higher relative to results presented in the research by *Pantelic et al. (2011)* in Simmental (3886 kg, 3,88%). If compare our with results of *Nistor et al. (2011)* for Romanian Simmental dairy cows in first (4311 kg, fat 3,92%, protein 3,20%) and second lactation (4977 kg, fat 3,90%, protein 3,19%), the average milk yield is higher, but milk fat and protein content are lower. The average milk yield was lower compare to those obtained by *Trivunovic et al. (2008)* in Simmental (6646 kg), and higher for milk fat content (3,82%). *Macciotta et al. (2002)* reported results with higher milk yield (6268 kg) and milk protein content (3,35%), and lower milk fat content (3,85%) for Simmental cows in 3-rd parity.

The study also analyzed the effect of fixed (systematic) factors on milk traits, and results are presented in Table 2.

Table 2. Effect of fixed factors on milk traits

Source of variability	d.f.	F-value				
		MY	FY	FP	PY	PP
Breeding region	11	35,83**	27,19**	6,92**	38,67**	31,53**
Calving year	3	4,95**	6,89**	1,05 ns	2,88*	5,10**
Calving season	3	9,37**	6,10**	2,15 ns	6,33**	6,71**
Lactation number	7	4,47**	4,57**	2,37**	4,58**	1,23 ns

d.f. – degree of freedom; ns – no significant * – P<0.05; ** – P<0.01
MY – milk yield; FY – fat yield; FP – fat percentage; PY – protein yield; PP – protein percentage

Breeding region had high significant effects on all observed milk traits. Calving year and season did not have significant effect only on fat content, while lactation number did not have significant effect only on protein content. The effect of systematic factors on milk yield in Simmental cows has investigated *Petrovic et al. (2009)* and found that, among others, breeding area (region) and calving season had statistically highly significant effect on milk traits. Also in other studies breeding region or farm usually have significant impact on milk performance traits due to different rearing methods, nutrition, housing, care, climatic conditions, age structure and herd size, as well as series of other influences related to work and farm management *(Petrovic et al., 2012; Radinovic et al. 2013),* also reported that the farm had a highly significant effect on all observed features, like calving year too, while season did not had effect only on milk fat content.

The estimates of systematic factors are important because they have a substantial share of total variations in milk production. *Stojic et al. (1995)* has found that farm, year and calving season high significant effect and induced 35,7% of total variations in milk production.

The estimates of genetic parameters such as heritability requires calculation of variance components, i.e. which of those fixed factors, next to genetic factors, have impact on the milk traits. Across variance components is possible to estimate the individual importance of different sources of variability, no matter if they come from the influence fixed or random factors.

Additive genetic, phenotypic and residual variances for milk yield, fat and protein yield and content, are shown in Table 3. Heritability's for these traits were calculated from the variance components, estimate using REML in animal model, and also shown in Table 3.

Table 3: Estimates of variance components and heritability

Traits	Variance component			h^2±S.E.
	V_A	V_R	V_P	
Milk yield, kg	121,26	99,25	220,51	0,55±0,01
Fat yield, kg	22,57	13,16	35,73	0,63±0,05
Protein yield, kg	17,34	17,17	34,51	0,50±0,01
Fat, %	0,17	0,36	0,53	0,32±0,05
Protein, %	0,04	0,07	0,11	0,37±0,05

V_A – additive variance; V_R – residual variance; V_P – phenotypic variance
h^2 – heritability; S.E. – standard error

Heritability, as a value that express and measure average additive gene effect, is one of the major characteristics of quantitative traits from the point of view of creation of genetically highly valuable cattle populations. Estimates of heritability for milk, fat and protein yields and content ranged from 0,32 to 0,63, respectively. Heritability for milk yield of 0,55 is higher than the one reported by *Nistor at al. (2011)* for Romanian Simmental in first and second lactation (first 0,144, second 0,183). *Pantelic et al. (2011)* reported heritability for Simmental of 0,487, *Nixon et al. (2009)* for Canadian Holstein (0,26). Higher heritability for milk yield in Holstein cow in Vojvodina stated *Radinovic et al. (2013)* with values 0,88 (milk yield), 0,92 (fat yield) and 0,25 (fat content). *Chauhan and Hayes (1991)* found lower heritability for fat (0,31) and for protein yield (0,25), as well as *Pantelic (2011)* who obtained heritability 0,455 for fat yield and 0,093 for fat content. *Toit et al. (1998)* found higher heritability for fat (0,57) and protein content (0,58) in South African Jersey.

Heritability indicates what percentage of total variation for a trait is the result of genetic differences between animals and it is an important factor in the rate of genetic change. The higher the heritability the greater the genetic control on the trait, and the more rapidly selection will result in genetic progress.

Conclusion

Milk production traits are determined by genetic and non-genetic factors. The values of heritability are specific to each population while the estimate is variable, both because of application of breeding program and prescribed methods of selection, and because of changes in management practices. Heritability values shows that the additive genetic variance has a significant proportion in considerable phenotypic variability of production traits of Simmental cows in Vojvodina, which is why it is justified to expect significant improvement of production traits of Simmental cows in Vojvodina through genetic selection. Consequently, breeding program for Simmental cows in Vojvodina include

production traits as most important component. Also, it is necessary to balance the breeding program by including functional and conformational traits. Genetic improvement of Simmental cattle in Vojvodina should be accompanied by appropriate management practices on dairy farms (nutrition, reproduction, health care, etc.).

Estimation of heritability as one of the basic genetic parameters is essential for setting breeding objectives about milk productions, as well as for the implementation of the breeding program. Obtained heritability values ranged from middle to high level, which is a good precondition for further work on the genetic improvement of milk production traits of Simmental breed in Vojvodina.

Acknowledgements

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Procena komponenti varijanse i heritabilnost za osobine mlečnosti krava simentalske rase u Vojvodini

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Rezime

Simentalska rasa, iako kombinovani tip, odnosno rasa dvojnog pravca proizvodnje (mleko i meso), daje odlične rezulate i u proizvodnji mleka. Proizvodne osobine (prinos mleka, mlečne masti i proteina, sadržaj mlečne masti i proteina) su osobine koje donose prihod u uzgoju mlečnih goveda; prinos mleka je jedna od ekonomski najznačajnih osobina u mlečnoj industriji, Upravo zato savremeni odgajivački programi za mlečne rase uključuju praćenje proizvodnih osobina. U Vojvodini je prisutna značajna fenotipska varijabilnost proizvodnih osobina mlečnih krava. Cilj ovog istraživanja je bio da se procene komponente varijanse i heritabilnost za osobine mlečnosti krava simentalske rase svih pariteta u Vojvodini, korišćenjem podataka iz izveštajne dokumentacije o kontroli produktivnosti mlečnih krava (zaključene laktacije) koji su dobijeni od Glavne odgajivačke organizacije (Poljoprivredni fakultet u Novom Sadu, Departman za stočarstvo), za 2013. i 2014. godinu. Obrađeni su podaci zaključenih laktacija za 4736 krava simentalske rase, za koje su izračunati deskriptivni statistički parametri. Uticaji fiksnih faktora su ispitani GLM metodom u programskom paketu Statistica 12. Genetske analize (procene komponenti varijanse i heritabilnosti) su izračunate

REML metodom u programskom paketu WOMBAT. Dobijen je prosečan prinos mleka od 5760 kg, prinos mlečne masti 224 kg, te sadržaj mlečne masti 3,88%; prinos proteina je 189 kg, dok je sadržaj proteina 3,28%. Od fiksnih faktora, region je imao visoko značajan uticaj na sve osobine mlečnosti, dok godina i sezona teljenja nisu imali značajan uticaj samo na sadržaj protein u mleku. Heritabilnost za osobine mlečnosti se kretala u rasponu od 0,32 (sadržaj mlečne masti) do 0,63 (prinos mlečne masti). Dobijene vrednosti heritabilnosti pokazuju da aditivna genetska varijansa ima značajan udeo u fenotipskoj varijabilnosti proizvodnih osobina krava simentalske rase u Vojvodini. Opravdano je očekivati poboljšanje proizvodnih osobina primenom savremenih metoda selekcije koje se pre svega zasnivaju na genetskim procenama grla i populacije.

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THE INFLUENCE OF ENVIRONMENTAL FACTORS ON THE OCCURRENCE OF GASTROINTESTINAL HELMINTHS OF GOATS IN SERBIA

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Abstract: Small ruminants production, among them goat production,play important role in providing economical income and high quality animal protein for diet especially for those who live in the rural area. Goats in this region are usually kept under semi-intensive (pasture/stable) condition. One of the main health problems in this kind of producing system is connected to infestation with parasites, which results in direct and indirect economical losses. Coprological and post-mortem examination, in a total of 910 fecal samples and 67 post mortem examined goats, revealed the presence of the following gastrointestinal helminths: *Teladorsagia* (*Ostertagia*) *circumcincta*, *O.trifurcata*, *O.ostertagi*, *Trichostrongylus axei*, *T.colubriformis*, *T. vitrinus*, *Nematodirus spathiger*, *N. filicolis*, *Haemonchus contortus*, *Marshallagia marshalli*, *Skrjabinema ovis*, *Bunostomum trigonocephalum*, *Chabertia ovina*, *Oesophagostomum venulosum*, *Cooperia curticei*, *C.oncophora* and *C.punctata*. The result of this study is a survey of sesonal dinamics of helminth species affecting goats in the region of Serbia, also an implication for development of strategic prevention and therapeutic mesures.

Key words: environmental factors, goats, helminths

Introduction

Goats population in Serbia had a drastical decrease after II WW, but in last decade were stared to anew increase of its population espacialy at mautntain areas *(Palić, 2001).* Today, goats are mostly breed in small flocks, usually often with sheep *(Jovanović et al., 2006)*. Pasture breeding make possible contact within eggs, larvae stages and intermediate host of parasites *(Vlassoff, 1982)* Those induce that there are no one goat without *parasites (Denev and Kostov, 1984; Vlassoff et al., 2001; Torina et al., 2004; Ardeleanu et al., 2007).* Examination of goats parasitoses were only sporadicly performed with the disruption of more than 30 years, when the goats were prohibited animals *(Vujić and Bošković, 1981; Vujić et*

al.,1991; Ilić,1981) When these extremely harmful laws repealed and re-started with breeding of goats, felt the lack of knowledge of parasitological situation among them. Experiences from around the world has shown that parasites have an important role in the pathology of goats and produce important productive loses *(Ilić, 1991; Ilić et al., 1991; Pavlović et al., 2003,).* From these reasoni in mind, during aplication of project we started with examination of parasitic fauna of goats at warious pats of Serbia. In our paper we presented results obtained during all years of research parasitofauna goats in different regions of Serbia performint like a part of project BT 31053.

Materials and Methods

The study about biodiversity and season distribution of gastrointestinal helminthes of goat's flocks were performed at spread Belgrade area, south-east and western parts of Serbia, hilly mountain area of Serbia and at Stara Planina. Examination was started in March 2010 and finished in October 2014 *(Pavlović et al., 2011a; 2012a,b,c; 2013a,b).*

During study we collected fecal samples at monthly intervals. A total of 4710 fecal samples originated from 221 flocks were analyzed. Examination was performed using standard coprological technique with saturated NaCl solution and sedimentation *(Euzeby, 1981).* Eggs per gram count (EPC) and degree of infection we assessed by McMaster technique where EPC of 50-700 eggs we treated like low rate of infection, to 1100 like moderate and up 1100 like high.

At same time a total of 267 animals 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 Anđelić-Buzadžić (2010).* Determination of adult helminthes and eggs of parasites were done by keys given by *Euzeby (1981)* and *Soulsby (1977).*

Results and Discussion

In our study we established the following genera of gastrointestinal helminths: *Trichostrongylus spp., Nematodirus spp., Ostertagia spp., Haemonchus spp., Chabertia ovina, Cooperia spp., Oesophagostomum spp., Marshallagia spp., Skrjabinema capre* and *Bunostomum spp.* The most prevalent genus was Trichostrongylus *spp.* The obtained value of intensity of infection measured by EPC were presented at Table 1

Table 1. Intensity of infection (EPC)

genera	Intensty of infection (EPC)		
	minimum	maximum	average
Trichostrongylus spp.	952	1026	989
Nematodirus spp.	977	1235	1106
Ostertagia spp.	300	622	461
Haemonchus spp.	548	672	610
Chabertia spp.	917	1021	969
Cooperia spp.	3233	4421	3872
Oesophagostomum spp.	512	626	569
Marshallagia spp.	521	643	582
Skrjabinema spp.	675	877	776
Bunostomum spp.	455	571	513

During our study, by post-mortem examination we established 19 gastro-intestinal helminth species: *Teladorsagia* (*Ostertagia*) *circumcincta*, *O.trifurcata*, *O.ostertagi*, *Trichostrongylus axei*, *T.colubriformis*, *T. vitrinus*, *Nematodirus spathiger*, *N. filicolis*, *Haemonchus contortus*, *Marshallagia marshalli*, *Skrjabinema ovis*, *Bunostomum trigonocephalum*, *Chabertia ovina*, *Oesophagostomum venulosum*, *Cooperia curticei*, *C.oncophora* and *C.punctata*. Percent of prevalence of each occurred species are presented in table 2.

Table 2. Helminth species, average prevalence (%) and intensity of infection

Parasites species	average prevalence (%)	intensity of infection	
		min	max
Teladorsagia (Ostertagia) circumcincta	92.73	23	955
Ostertagia trifurcata	91.53	8	52
Ostertagia ostertagi	28.33	11	64
Trichostrongylus axei	99.30	2	258
Trichostrongylus colubriformis	88.67	3	4457
Trichostrongylus capricola	61.09	7	921
Trichostrongylus vitrinus	21,37 *	5	231
Nematodirus spathiger	100.00	17	6361
Nematodirus filcolis	32.31	96	270
Haemonchus contortus	86.26	12	104
Marshallagia marshalli	28.77	18	188
Skrjabinema caprae	12.19	4	17
Skrjabinema ovis	08.77	3	19
Bunostomum trigonocephalum	15.28	8	232
Chabertia ovina	61.70	2	6
Oesophagostomum venulosum	24.89	8	14
Cooperia curticei	58.52	11	97
Cooperia oncophora	09.29 *	6	31
Cooperia punctata	05.26 *	9	42

- found only at west Serbia

The epidemiology of the helminth parasitic diseases therefore depends on factors such as the infection pressure in the environment and the susceptibility of the host species (or individual). Furthermore, the availability of large numbers of susceptible definitive and intermediate hosts will increase the parasites' ability to reproduce and result in high parasite abundance *(Vlasoff et al., 2001).*

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. Development occurs within the faecal mass, the eggs embryonate and hatch into first-stage larvae (L1), which in turn moult into second-stage larvae (L2), shedding their protective cuticle in the process *(Vlasoff et al., 2001; Pavlović and Anđelić-Buzadžić, 2011b).* During this time the larvae feed on bacteria. The L2 moult into third-stage larvae (L3), but retain the cuticle from the previous moult. The L3 constitute the infective stage, and these migrate onto surrounding vegetation where they become available for ingestion by grazing sheep and goats.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 *(Pavlović et al., 2012d; Žugić, 2013).* These include sunlight, temperature, rainfall, humidity and soil moisture. At climate condition which are present in examined areas. The climate of Serbia is mostly temperate continental with steppe character. The temperature region in Serbia, which has a mean annual temperature of between 11^{o}C and 12^{o}C are plains in the north and northeast, the Morava and the areas with an altitude of less than 500 m. The average annual amplitude of temperature variations in the eastern and northeastern Serbia is 23-24°C. The average temperature of air in mountainous areas and in the highlands are amplitude from -4°C to 18°C. In lowland areas, river basins and Negotinska Krajina temperature variations is between 0 and -2°C in winter to 22°C in summer time. Annual precipitation regime has two maximums, primary in late spring and secondary, in late autumn; winter and summer, the drought periods. Annual precipitation sums rise in average with altitude. In lower regions annual precipitation height range in the interval from 540 to 820 mm. The dynamics of the first occurrence of established species of gastro-intestinal strongilida was as follows:

- In March have occurred *Ostertagia spp.* and *Trichostrongylus spp.*
- In May, the observed infection with *Nematodirus spp, .Bunostomum spp.* and *Chabertia spp.* (*ovina*)
- In June was the first record of *Skrjabinema spp*;
- In July were established eggs of *Haemonchus spp.* (*contortus*) and *Cooperia spp.*
- In November showed the presence of *Marshallagia spp*

At the beginning of our research, conducted in March, the real extent of gastrointestinal infections strongilidae was 83.33%, after which he soon reached a level of 100% in the same way and moved to the end of follow-up period. Extensity of infection established genera gastrointestinal strongilidae was different. The distribution of parasites of the genera *Ostertagia, Trichostrongylus* and *Nematodirus* was reached during the monitoring period almost the maximum level. The distribution of species within the established genera also varied. Within the genus *Ostertagia* most abundant were dominated by *Ostertagia circumcincta* and *O.trifurcata*. Prevalence of infection with *Ostertagia ostertagi* and *Ostertagia occidentalis* was higher during the colder periods of the year.Among the species of the genus *Trichostrongylus* was the most prevalent *Trichostrongylus colubriformis*. Extensity of infection with *Trichostrongylus axei* and *T.vitrinus* varied, without any regularity. Extensity of infection with *Nematodirus filicollis* and *N.spathiiger* demonstrated a tendency to increase and leveled off at the highest level of the whole study period. During our examination *Cooperia punctata, Cooperia oncophora* and *Trichostrongylus vitrinus* was established at goats only at western part of Serbia.

As the parasitic fauna of sheep and goats identical, is possible comparison with the research helminths of sheep in some areas Serbia. Compared our results with that examination we were concluded that season distribution were different with our results. During those examination at East Serbia (including Šara Mountain) strongillidae of genera *Haemonchus*, *Ostertagia,*and *Nematodirus* was most abundant at spring and summer and strongillidae of genera *Trichostrongylus* and *Marshallagia* during outman and winter period At mountain area of Serbia (Sjenicko-Pesterski Highland, Dimitrovgrad, Tutin and Zlatar mountain) strongillidae of genera *Ostertagia* and *Nematodirus* was most abundant at April, *Nematodirus* and *Cooperia* species and *Haemonchus contortus* during spring and summer and strongillidae of genera *Trichostrongylus,* and *Marshallagia* during outman and winter period *(Lepojev, 1965; Pavlović, 1972; Jovanović et al., 1991, Pavlović et al., 1995, 2009).*

Similar results were obtained during the studies in neighboring countries. In Macedonia *Iliev (1974)* and later *Georgievski at al. (1991)* occured *Trichostrongylys* specis at spring months, and *Marshallagia marshalli, Chabertia ovina* and *Oesophagostomym* species only at winter period. Simillary results were established in Montenegro by *Karanfilovski (1991),* in Bulgaria by *Denev and Kostov (1984)* and in Romania by *Ardelaeanu et al. (2007).*

Conclusion

In order to achievee better goat productive traits under semi-intensive breeding condition, an integrated approach is necessary. The approach must contain all aspects of goat keeping, feeding, breeding and disease prevention, as well as pasture management. However, since the parasitic infections are in majority sub clinical this problem is not played due attention to in Serbia. 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.

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Uticaji životne sredine na pojavu gastrointestinalnih helminata kod koza u Srbiji

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Rezime

Uzgoj malih preživara, pogotovu koza zauzima sve veće mesto u proizvodnji mesa i mleka, naročito ruralnim područjima. Koze se tu uzgajaju pretežno u ekstenzivnim uslovima i jedan od najznačajnih zdravstvenih problema prouzrokuju su usled infekcije parazitima. Pašnim načinom držanja koze dolaze u dodir sa jajima i prelaznim domaćinima mnoštva parazitskih vrsta tako da su ove infekcije stalan pratilac kozarske proizvodnje koji uzrokuje indirektne i direktne ekonomske gubitke. U okviru projekta BT 31053 izvršili smo koprološki pregled 910 koza i obdukciju 67 životinja da bi ustanovili vrste i zastupljenost gastrointestinalnih helminata kod njih. Tokom ovih pregleda ustanovljene su sledeće vrste parazita: *Teladorsagia* (*Ostertagia*) *circumcincta*, *O.trifurcata*, *O.ostertagi*, *Trichostrongylus axei*, *T.colubriformis*, *T. vitrinus*, *Nematodirus spathiger*, *N. filicolis*, *Haemonchus contortus*, *Marshallagia marshalli*, *Skrjabinema ovis*, *Bunostomum trigonocephalum*, *Chabertia ovina*, *Oesophagostomum venulosum*, *Cooperia curticei*, *C.oncophora* and *C.punctata*..Rezultati studije pokazuju da je sezonska dinamika vrsta helminata kod

koza bila u direktnoj zavisnosti od uslova životne sredine što će pomoći u sagledavanju strategije potrebne za preventivu i suzbijanje parazitskih infekcija.

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EFFECT OF DIFFERENT HOUSING SYSTEMS ON QUALITY OF TABLE EGGS

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Original scientific paper

Abstract: In recent years consumers pay more attention to the housing system in which the eggs are produced, so there is a relatively small market niche for organic products in Serbia. Most of the consumers consider that eggs from organic production are better for their health compared to conventionally produced eggs. This attitude of consumers is usually based on the belief that there is a significant difference between organic and conventional eggs in their chemical composition, The aim of this work was determine the chemical composition and the internal and external quality of eggs which are present on a Serbian market and that originate from different housing systems – conventional cages, free range and organic production. The results showed that there were significant differences ($P<0.01$) in fat content in the yolk, which was higher in organic eggs. Housing system significantly influenced the egg mass, shape index, cleanness of egg shell, Haugh units and yolk color in favor of eggs from conventional and free range production. Shell breaking force was the lowest in eggs from conventional production.

Key words: housing system, eggs, quality, chemical composition

Introduction

In recent years consumers pay more attention to the housing system in which the eggs are produced. Although the legislation concerning animal welfare has been changed and the ban of the using conventional cages had been postponed until 2020. many producers started to change housing systems towards enriched cages, free range and organic production.

Organic livestock production in Serbia is still at the beginning, despite of the fact that the interest of consumers for buying so called „healthy food“is growing. However, most of the consumers in Serbia are not willing to pay much higher price for the organic or free range eggs. The results of questionnaire on

sample of 720 persons showed that in case of choice only 9% of interviewees would purchase eggs from conventional cages *(Rodić et al., 2010a).* Majority would choose eggs from free range system (51.2%) or organic production (26.4%), but they are not willing to pay more than 10-20% higher price. This is obviously one of the reasons why the alternative production systems are not introduced into poultry production in a large scale.

However, there is a small market niche for organic products in Serbia. Most of the consumers consider that eggs from organic production are better for their health compared to conventionally produced eggs, but they don't have the exact explanation why *(Rodić et al., 2010b).* This attitude of consumers is usually based on the belief that there is a significant difference between organic and conventional eggs in their chemical composition, although there is not enough scientific evidence for that. There is no evidence that the cholesterol content of eggs is lower in organic than in regular eggs, although the fatty acid (FA) profile may be more favorable *(Samman et al., 2009; Terčić et al., 2012).*

The aim of this work was determine the chemical composition and the internal and external quality of eggs which are present on a Serbian market and that originate from different housing systems – conventional cages, free range and organic production.

Material and Methods

Sampling of eggs was carried out on a certificated organic farm and conventional and free range eggs were taken from the market. In a sample of 30 eggs of each housing system the internal and external egg quality traits were examined. Egg weight was measured on a precision scale. Shell color was assessed by points form 1 (pale) to 5 (dark brown). Shell cleanness was assessed by points on a scale from 0 (clean) to 3 (very dirty). Shell breaking force was determined by instrument Egg Force Reader (Orka Food Technology Ltd, Israel). Yolk color was determined using the Roche fan. Albumen height was measured with a tripod micrometer. On the basis of egg mass (M) and albumen height (H), Haugh units were calculated according to formula $HU=100\log (H+7{,}57-1{,}7M^{0.37})$.

Basic chemical composition of eggs was determined in the Laboratory for feed quality control on Faculty of Agriculture in Novi Sad. Protein content was determined by Kjeldahl method and fat content was determined using the Soxlet method. Statistical analyses were done in program STATISTICA 12 (Stat Soft, 2015). Most of the parameters were analyzed using ANOVA with Duncan's post-hoc test.

Results and Discussion

The results of this work confirmed that there are differences in chemical composition of eggs from organic compared to conventional and free range production (table 1).

Table 1. Basic chemical composition of eggs from different housing systems

Nutrient, %	Housing system		
	Cages	Free range	Organic
Egg white			
Crude proteins	10.74	10.17	10.09
Crude fat	0.09	0.07	0.03
Egg yolk			
Crude proteins	16.08	15.43	16.39
Crude fat	25.93^{B}	25.59^{B}	28.46^{A}

$^{A-B}$ Values in the same raw without common superscript are significantly different (P<0.01)

The results showed that there was no significant difference in the protein content in eggs from different housing systems. On the contrary, *Minelli et al. (2007)* stated that organic egg yolks had a significantly higher content of protein compared to conventional eggs. Regarding the fat content, significant difference was established in fat content in yolk between organic eggs (28,46%) and conventional eggs (25,93%) or free range eggs (25,59%). The same finding was reported by *Perić et al. (2015).* However, most of the research on nutritional composition of organic eggs has been directed at the fatty acid composition and level of cholesterol rather than fat content *(Milinsk et al., 2003, Samman et al., 2009*).

Based on the previously published results it could be stated that there are differences in basic chemical composition of eggs from different housing systems, but the effect is not consistent and can be achieved by the use of appropriate dietary ingredients. The main problem in so called “market research” is that researchers who purchase eggs at a retail outlet have no control over the composition of the diets fed to the birds *(Hidalgo et al., 2008)*. Consequently, the most useful research reports are those in which the feed composition was controlled and the composition of the diet published.

Table 2. External and internal characteristics of eggs from different housing systems (mean ± SD)

Egg quality traits	Housing system		
	Cages	Free range	Organic
Egg weight, g	61.40 ± 1.35^{B}	62.95 ± 1.27^{A}	59.23 ± 5.35^{C}
Shape index	77.05 ± 2.33^{a}	74.9 ± 3.67^{b}	74.9 ± 2.51^{b}
Shell color, points	2.85 ± 0.75	3.15 ± 0.67	2.55 ± 1.24
Shell cleanness, points	1.30 ± 0.65^{B}	1.05 ± 0.22^{B}	1.75 ± 0.72^{A}
Shell breaking force, kg	3.67 ± 0.86^{B}	4.24 ± 0.69^{A}	4.46 ± 0.61^{A}
Shell thickness, 0.01 mm	35.82 ± 2.94	37.06 ± 2.54	35.65 ± 2.64
Yolk color (Roche)	11.59 ± 1.12^{ab}	12.06 ± 0.92^{a}	10.89 ± 1.04^{b}
Haugh units	85.44 ± 6.85^{A}	69.5 ± 8.3^{B}	69.24 ± 7.96^{B}

Values in the same raw without common superscript are significantly different $^{A-C}$ ($P<0.01$) $^{a-b}$ ($P<0.05$)

Housing system significantly affected the egg quality. Eggs from organic system had the lowest weight and the eggs from free range system the highest. The same result was reported by *Minelli et al. (2007)* and *Đukić Stojčić et al. (2015)* while *Terčić et al. (2012)* didn't get any differences in egg mass between organic and conventional eggs. It is questionable if this trait had to be compared in market studies, since hens are not the same age and breed.

Interestingly, eggs from conventional cages had significantly different egg shape compared to the other groups – they were more round. *Đukić Stojčić et al. (2015)* reported similar finding. However this trait as well as the shell color does not influence the internal quality of the egg such as flavor, nutritional composition, or cooking characteristics. As expected the shell cleanness was the worst in organic system because the hens spent more time outside the barn compared to other two systems.

A significant difference was established in shell quality. Shell breaking force was the lowest in eggs from conventional cages and the highest in organic eggs. On the contrary *Minelli et al. (2007)* reported significantly lower eggshell breaking strength in organic eggs. *Rizzi et al. (2006)* and *Mugnai et al. (2009)* found thicker shells in organic eggs in comparison to conventional eggs. *Pavlovski et al. (2001)* confirmed that housing system affects the shell quality. They detected ticker shells in barn eggs compared to free range eggs.

Yolk color significantly differed between housing systems. Yolk form organic eggs were paler compared to other two systems which makes sense since the use of synthetic pigments is not allowed in organic production. Similar results were reported by *Hidalgo et al. (2008), Minelli et al. (2007)* and *Terčić et al. (2012).* They all agree that use of feed with synthetic pigments results in higher yolk yellowness.

Haugh units were significantly lower in organic and free range eggs. Also *Patterson et al. (2001),* analysing different shell egg types, observed lower HU values in organic eggs than in cage and other specialty eggs in the USA market. *Đukić Stojčić et al. (2015)* also established that Haugh units were higher in eggs from conventional compared to organic production. *Terčić et al. (2012)* found lower value of Haugh units in organic eggs, but the difference was not significant. The causes of the low HU values in organic eggs have to be further investigated since albumen quality could be determined not only by egg freshness but also by other factors such as hen age and genotype and dietary ingredients *(Hidalgo et al., 2008).*

Conclusion

This work confirmed that there are differences in the nutritional quality of eggs from organic compared to conventional production. The difference was the most prominent in fat content in the yolk, which was significantly higher in organic eggs. Significant effect of housing system was established on the internal and external egg quality traits. Generally, eggs from conventional and free range production showed better egg quality compared to the eggs from organic production. Housing system significantly influenced the egg mass, shape index, cleanness of egg, Haugh units and yolk color in favor of eggs from conventional and free range production. Shell breaking force was the lowest in eggs from conventional production.

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Efekat različitih sistema držanja na kvalitet konzumnih jaja

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Rezime

Poslednjih godina potrošači sve više obraćaju pažnju na način držanja kokoši nosilja, tako da i u Srbiji postoji relativno mala tržišna niša za organske proizvode. Većina potrošača smatra da su jaja iz organske proizvodnje zdravija u odnosu na konvencionalna. Ovaj stav potrošača uglavnom je baziran na verovanju

da postoji značajna razlika u hemijskom sastavu između organskih i konvencionalnih jaja. Cilj ovoga rad bioje da se utvrdi hemijski sastav i kvalitet konzumnih jaja iz različitih sistema držanja – konvencionalni kavezi, jaja sa ispusta i organska proizvodnja. Rezultati su pokazali da postoji značajna razlika ($P<0.01$) u sadržaju masti u žumancetu koji je bio veći kod jaja iz organske proizvodnje u odnosu na druga dva sistema. Sistem držanja značajno je uticao i na osobine kvalieta jaja, kao što su masa jaja, čistoća ljuske, indeks oblika, Hogove jedinice i boja žumanca koji su bili bolji kod jaja iz konvencionalne proizvodnje i sa ispusta u odnosu na organsku proizvodnju. Jaja iz konvencionalne proizvodnje imala su najnižu silu loma ljuske.

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POSTER SECTION II

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INFLUENCE OF CAGE SYSTEM ON LAYING HEN PRODUCTIVITY AND ON ECONOMY ASPECTS OF EGG PRODUCTION

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Abstract: In order to determine cage system influence on laying hen production traits and economy aspects of egg production total number of 68000 Lohmann Brown laying hens were used. First group (C – Control, 34000 birds) of seventeen week old hens was put in Conventional four floor cages (4-6 birds per cage), second (E – experimental, 34000 birds) group was put in five floor furnished cages (up to 30 birds per cage). Egg production period for both groups was 53 weeks (from 19th till 72nd week of age). During the production cycle total number of produced eggs per hen was 311.60 (C cage) and 312.52 eggs (F cage), weekly production per hen was 5.88 and 5.90 eggs. Average daily food consumption per hen for entire production cycle was 129.43 g (C cages) and 129.66 g (F cages), feed consumption per egg was 163.80 g and 158.09 g. In the period from 19th till 71st week total number of died or removed hens was 2891 or 8.46% (C cage) and 2431 or 7.10% (F cage). Determined differences in the terms of egg production, laying intensity and feed consumption were not statistically significant ($P>0.05$), while in the terms of mortality rate they were statistically significant ($P<0.05$). Economy coefficient was similar for both flocks and it was 1.61 for C cage and 1.60 for F cage, while profitability rate for hens kept in conventional cages was 60.71% while for hens kept in furnished cages rate was 60.56%.

Key words: conventional cages, furnished cages, consumption eggs, productivity, economic aspect.

Introduction

Keeping of the commercial flock in conventional cages has advantage in the terms of disease prevention, better hen control and maximal automatisation during the production cycle is possible. Disadvantage is that that hens have lack of moving space and they get injured more frequently. When it comes to furnished cages, advantage is that hens have more moving space. They also provide far more enriched behavioral repertoire where hens can use nest site and perches.

Production results of hens kept in furnished cages can be at the production levels of conventional cages only if they are properly constructed and if proposed breeding technology is strictly followed for the hen breed that is being kept in the cage. This was confirmed in the research conducted by the *Radović (2011)* which determined similar production results for hens kept in furnished (enriched) and conventional cages.

It is well known that since 2012 the use of conventional cages for laying hens was banned in EU directive (EU Council Directive 99/74/EC), where it is stated that laying hens can be kept in cage system but only in furnished cages or in some type of alternative system. Due to relatively short period that furnished cages were used, majority of authors were conducting research of breeding in conventional or some alternative type of cage use, while relatively small number of authors researched influence of furnished cages on productive performances of laying hens and egg quality *(Petrović et al., 1981, 1982; Hartman and Heil, 1985; Stolić et al., 1994; Perić et al., 2001, 2007; Rajičić, 2003; Guesdon and Faure, 2004; Basmaciuolu and Ergul, 2005; Thomas and Ravindran, 2005; Elson and Croxall, 2006; Senčić and Butko, 2006; Rajičić et al., 2007; Pohle and Cheng, 2009a,b; Pandurević, 2011)* and economy factors of consuming egg production *(Crnčan et al., 2014)*.

Therefore main goal of this research has emerged. Comparative analysis of production results (laying intensity, mortality and feed consumption) of Lohmann Brown laying hens reared in conventional and furnished cages. Moreover economy parameters (economy value and profitability) were determined for both types of cages within one productive cycle which lasted 53 weeks.

Material and Methods

With the goal to determine influence of cage system on productive traits and on production economy aspects total number of 68000 Lonmann Brown laying hens were used. First group was control (C) group where 34000 seventeen week old laying hens were put in facility with four floor conventional cages, second group, experimental (E) group where the rest (34000) of seventeen week old hens

were put in facility with furnished five floor cages. Young hens of both groups were kept in cage system for chick rearing till they reached 17th week. Upon reaching 17th week hens were moved in to two separated facilities where they were reared in conventional (4-6 birds) and furnished (up to 30birds) cages. Each facility was inhabited with 34000 hens, and both flocks started laying eggs by the end of 18th week. In the 18th week laying intensity and egg weight were very low, and that is why, for both flocks as first egg production week 19th week was taken as starting week for analysis, even though it's slightly earlier than what technology normative propose for that hen breed.

During the rearing of both commercial flocks breeding technology which was recommended by Lonmann Brown selectioners was used. All technological phase of production cycle were automatically regulated, hens were fed per will (*ad libitum*) with complete mixtures containing needed amount of energy and proteins.

Egg production period lasted 53 weeks (19th till 72nd week of age). In that period mortality rate, laying intensity and feed consumption per hen and per produced egg were monitored. Based on income structure (income from egg sale and extracted hens) and cost structure (entry value for flock replenishing, for feed mixtures, water, floor cover, electricity, veterinary expenses, facility and equipment amortization, cleaning and disinfection, insurance, egg containers and other expenses) total income (T.I.) and total expenses (T.E.) were determined. Financial result for both flocks was calculated (F.R. =T.I.–T.E.), economic value (E.V.=T.I./T.E.) and profitability (P=(F.R./T.E.)x100) of consumption eggs production.

Usual statistic indicators were calculated for all parameters: average values, arithmetic mean error, standard deviation and variation coefficient, obtained differences between some indicators were tested with T-test. Statistic data rendering was done in SAS/STAT (2000).

Results and Discussion

During the production cycle special attention was given to noting and monitoring the results which are related to laying intensity, feed consumption and mortality for both flocks. Average values, variability and significance of determined differences are shown in table 1.

Table 1. Average values, variability and difference significance of productive traits of hens reared in conventional and furnished cages.

Parameters	Cage model	$\bar{X}$ ±SEM	S	C.V.	d	Significance
Egg per hen/week	C	5.88±0.13	0.94	15.99	-0,02	ns
	F	5.90±0.11	0.84	14.24		
Laying intensity (%)	C	83.99±1.84	13.41	15.97	-0,25	ns
	F	84.24±1.65	12.00	14.25		
Daily food consumption per hen (g)	C	129.43±0.88	6.44	4.98	-0.23	ns
	F	129.66±0.88	6.40	4.94		
Food consumption per egg (g)	C	163.80±8.21	59.77	36.49	5.71	ns
	F	158.09±4.49	32.71	20.69		
Mortality (absolute)	C	54.55±2.84	20.84	38.20	8.68	*
	F	45.87±2.44	17.79	38.78		
Average weekly mortality (%)	C	0.16±0.01	0.06	38.20	0.03	*
	F	0.13±0.01	0.05	38.56		

$^{ns}P>0,05$; $^{*}P<0,05$.

During the production cycle (53 weeks) total number of produced eggs per hen was 311.60 (C cage) and 312.52 eggs (F cage), weekly production per hen was 5.88 and 5.90 eggs (table1). Determined difference regarding the average weekly egg production (-0.02 eggs) per hen was not statistically significant (P>0.05). Moreover data in table 1 shows that absolute and relative variation regarding the average weekly egg production per hen and laying intensity for hens reared in conventional and furnished cages was at satisfying level.

Average daily food consumption per hen for entire production cycle was 129.43 g (C cages) and 129.66 g (F cages), feed consumption per egg was 163.80 g and 158,09 g (table1). Conventional cages had lower daily food consumption per hen, while in furnished cages food consumption per egg was lower. Determined differences regarding these parameters were not statistically significant (P>0.05). Moreover data from table 1 shows that variability regarding the feed consumption per produced egg compared to daily feed consumption per egg was significantly higher for hens in reared in both cage types.

Relative mortality rate of the hens per weeks during the production reared in conventional cages was between 0.03% and 0.22%, while for hens reared in furnished cages was between 0.02% and 0.19%, however it has to be mentioned that variability during the production cycle for both cage type hens was very high and value of variation coefficient was over 38%. In the period from 19th till 71st week total number of died or removed hens was 2891 or 8.46% (C cage) and 2431 or 7.10% (F cage). Hens reared in conventional cages compared to hens reared in furnished cages had, averagely per weeks, higher mortality rate by 0.03%.

Determined differences regarding the mortality rate (table1) were statistically significant (P<0.05).

In comparison with the results by *Guesdon and Faure (2004)* considering the egg production in conventional and furnished cages both analyzed flocks expressed higher egg production, however they also determined that cage system had no significant influence on laying hens productivity. Significantly higher laying intensity of hens reared in conventional furnished cages was determined by *Elson and Croxall (2006)*. Significantly lower egg production per hen (K cages -260 eggs; O cages 272 eggs) was determined by *Radović (2011)*, but production cycle was shorter than 52 weeks. Similarly *Senčić and Butko (2006)* determined that Lohmann Brown hens produced 295 eggs in 52 weeks in conventional cages, while hens kept in open floor system produced 266 eggs. Very similar average laying intensity for 53 weeks (83.83%) was determined by *Pandurević (2011)*. Other authors who researched this subject *(Petrović et al., 1981, 1982; Hartman and Heil, 1985; Stolić et al., 1994; Perić et al., 2001, 2007; Rajičić, 2003; Basmaciuolu and Ergul, 2005; Thomas and Ravindran, 2005; Rajičić et al., 2007; Pohle and Cheng, 2009a,b)* determined for different genotypes of hens reared in conventional cages slightly and significantly lower laying intensity which was between 59.65% and 88.18%.

Fact that cage type (conventional and furnished) does not have influence on daily feed consumption per hen was determined by *Elson and Croxall (2006)*, even though they determined significantly lower daily feed consumption in some phases of production cycle. Similar feed consumption during the Lohmann Brown hens rearing (where production cycle lasted 53 weeks) in conventional cages was determined by *Pandurević (2011)* where average daily feed consumption was per hen 126.97 g, and per produced egg 151.47 g.

Guesdon and Faure (2004) have determined statistically very significant (P<0.001) mortality rate for hens from 18^{th} till 70^{th} week of age (52 weeks of egg production) kept in conventional cages compared to hens kept in furnished cages. Mentioned authors reared hens in two conventional cage types (S6 i S5) and two models of furnished cages (F15 i F7), where they determined following mortality rates: 21% - S6, 17% - S5, 11% - F15 i 10% - F7. *Elson and Croxall (2006)* also determined higher mortality rate for hens reared from 16^{th} till 70^{th} week of age in furnished cages (<8%), compared to hens reared in conventional cages (<6%). Lowest mortality rate of laying hens (5.8%) reared in furnished cages was determined by *Radović (2011)*, and lowest mortality rate for hens kept in conventional cages (5.5%) *Senčić and Butko (2006)*. Authors who conducted research regarding the productivity and mortality rate of hens reared in conventional cages obtained very variable results an mortality depending on hen hybrid and longevity of production cycle was between 4.30% and 11.11%. To be

exact for Lohmann Brown hens reared in conventional cages from 17th till 72nd week of age mortality rate of 8.18% and from 20th till 72nd week mortality rate of 8.07% was determined by *Pandurević (2011)*.

Based on total income structure and total expenses structure represented in calculation in table 2 economy indicators were determined (financial result, economy value, and profitability) which represents one of very significant parameters for laying hen rearing in conventional and furnished cages.

Tale 2. Calculation of egg production in conventional and furnished cages

Parameters	Cage model	Value
Total income (RSD)	C	169822237.50
	F	170391912.50
Total expenses (RSD)	C	106050233.00
	F	106503793.00
Financial result (RSD)	C	64377967.00
	F	64503247.00
Economy value	C	1.61
	F	1.60
Profitability	C	60.71
	F	60.56

Income structure for both flocks was determined by income from egg sale and from extracted hens (table 2). Hens reared in conventional cages obtained total income of 169822237.50 RSD, while hens reared in furnished cages obtained total income of 170391912.50 RSD. Total expense for C cages was 106050233.00 RSD and 106503793.00 RSD F cages. Data from table 2 also shows that financial gain for hens reared in conventional cages was 64377967.00 RSD, and for hens reared in furnished cages was 64503247.00 RSD. Economy value was similar for both flocks 1.61 for C cages, and 1.60 for F cages, while profitability rate for hens kept in conventional cages was 60.71% while for hens kept in furnished cages 60.56%.

From above mentioned it can be concluded that production results for hens reared in furnished cages can be at the level of results for hens reared in conventional cages, under the condition that cages are properly constructed and that rearing technology which is proposed for light line hen hybrid is strictly applied. However, economic value and profitability for producing consumption eggs and rearing hens in conventional and furnished cages can be better (unstable market), which is being shown by research conducted in Croatia where for this type of rearing economy value coefficient was 1.75 and profitability rate 75.33%. Significantly lower economy value (E=1.04) but for production of consumption eggs from organic farms was determined by *Crnčan et al. (2014)*.

Conclusion

Based on conducted research it can be said that both flocks of laying hens reared in conventional and furnished cages have obtained satisfying production results which were within the limits proposed by technology normatives for the breed. Besides, results obtained during the research and their comparision with recommendations (EU directive) and recommendations regarding the minimal healthcare protection of laying hens, point out that rearing hens from welfare aspect is better in furnished cages than in conventional cages which is proven with significantly higher mortality rate in conventional cages. Moreover, based on income and expence structure it can be said that hens kept in conventional and furnished cages obtained good financial result. However, one has to bear in mind economic justification of specific production type because investment in the equipment is much higher and utilization of facility capacity is much lower for hens reared in furnished than in conventional cages.

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Uticaj kaveznog sistema na produktivnost kokoši nosilja i na ekonomske aspekte proizvodnja jaja

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Rezime

Da bi se utvrdio uticaj kaveznog sistema na proizvodne osobine nosilja i ekonomske aspekte proizvodnje jaja, korišćeno je u istraživanju ukupno 68000 Lohmann Brown kokoši nosilja. Prva grupa (C - kontrola, 34000 nosilja) uzrasta od sedamnaest nedelja je držana u konvencionalnim četvrorospratnim kavezima (4-6 nosilja po kavezu), druga grupa (E - eksperimentalna, 34000 nosilja) je držana u pet podnih kaveza (do 30 nosilja po kavezu). Period/trajanje proizvodnje jaja za obe grupe bio je 53 nedelje (od 19. do 72. nedelje starosti). Tokom proizvodnog ciklusa ukupan broj proizvedenih jaja po kokoši je bio 311,60 (C kavez) i 312,52 jaja (F kavez), nedeljna proizvodnja po kokoši je bila 5,88 i 5,90 jaja, respektivno. Prosečna dnevna konzumacija hrane po kokoši za ceo proizvodni ciklus je 129,43 g

(C kavezi) i 129,66 g (F kavezi), potrošnja hrane po jajetu je bila 163,80 g i 158.09 g. U periodu od 19. do 71. nedelje, ukupni broj umrlih ili uklonjenih nosilja je bio 2891 ili 8,46% (C kavez) i 2431 ili 7,10% (F kavez). Utvrđene razlike u pogledu proizvodnje jaja, intenziteta nošenja i potrošnje hrane nisu bile statistički značajne (P>0,05), dok je u pogledu stope mortaliteta utvrđena statistički značajna razlika (P <0,05). Ekonomski koeficijent bio je sličan za oba jata i to 1,61 za C kavez i 1,60 za F kavez, stopa profitabilnosti za nosilja držane u konvencionalnim kavezima bila je 60,71% dok je za kokoške držane u poboljšanim kavezima bila 60,56%.

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EFFECT OF DIETARY PROTEASE SUPPLEMENTATION ON CARCASS WEIGHT AND DRESSING PERCENTAGE OF BROILERS

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Abstract: This paper presents research results on the effect of protease supplementation of reduced crude protein broiler diet on carcass weight and dressing percentage of two broiler genotypes: fast-growing Cobb 500 and slow-growing Master Gris. Complete feeds for broilers in experimental groups E-I and E-II contained 4 and 6% less crude protein than the control (C), and were supplemented with protease (Ronozyme Pro Act) at a concentration of 0.2 and 0.3%, respectively. At 49 days of age i.e. at the end of the experiment, 10 male and 10 female broilers of both hybrids were randomly selected from each experimental group and slaughtered. Upon slaughter, conventionally dressed, ready-to-roast and ready-to-grill carcass weights and abdominal fat weight were measured. These data and body weight at slaughter were used to calculate dressing percentages and abdominal fat percentage. The results showed a significant effect of genotype and no effect of dietary treatments on carcass quality traits.

Key words: broilers, protease, dressing percentage, abdominal fat.

Introduction

Broiler meat quality is dependent on a large number of factors, primarily genotype and nutrition. Modern broiler hybrids, fed complete feeds containing all necessary nutrients, exhibit a very rapid growth rate, resulting in high amounts of meat produced over a relatively short period of time. As reported by *Leeson (2007)*, modern broiler hybrids are characterized by a very high growth rate and low feed conversion, while metabolic diseases, leg problems and increased fat deposition are common occurrences. One of the most important nutritional requirements for optimum animal performance is to provide adequate dietary protein levels (*Bregendahl et al., 2002*). Today, broiler feeds are mostly based on

soybean meal and full-fat soybean grits as primary protein feedstuffs, but due to GMO contamination problems, alternatives to these feedstuffs as protein sources have been increasingly investigated in recent studies *(Meluzzi et al., 2009)*.

Many researchers *(Fidelis et al., 2010; Angel et al., 2011; Frietas et al., 2011)* have studied the effect of protease supplementation of broiler diets containing reduced amounts of plant feedstuffs, primarily soybean.

The objective of this study was to evaluate whether reducing the amount of soybean meal in broiler diets supplemented with protease has an effect on carcass weight, dressing percentage, and abdominal fat weight and percentage in two broiler hybrids.

Materials and Methods

A total of 300 day-old Cobb 500 broilers were allocated to three groups-boxes each containing 100 birds. The slow-growing Master Gris hybrid included 300 day-old broilers assigned to three groups with 100 broilers. Stocking density was 10 birds/m^2. The broilers were randomly grouped, giving a random ratio of male to female birds across groups. The chicks had free access to water and feed, and a 24-hour photoschedule was applied. Ad libitum feeding was used.

A three-stage feeding program was used, including starter (0-21 days), grower (22-42 days) and finisher stages (42-49 days).

One group of broilers served as the control-C (fed normal nutrient levels, in accordance with broiler requirements during certain fattening stages), and the other two groups were experimental groups E-I and E-II (fed diets with crude protein levels reduced by 4% and 6% and supplemented with 0.2% and 0.3% protease, respectively). Feed ingredients (used across feeding stages and experimental groups) of diets are presented in Table 1.

A protease preparation commercially called Ronozyme ProAct (produced by DSM, The Netherlands) was used in the fattening trial.

Table 1. Ingredients of experimental diets for fattening chickens[1]

Ingredient, %	Starter stage (1 to 21 d)			Grower stage (22 to 42 d)			Finisher stage (43 to 63 d)		
Treatments	C	E-1	E-2	C	E-1	E-2	C	E-1	E-2
Maize	52.49	54.92	56.26	63.15	65.28	66.34	68.62	70.60	71.59
Soybean meal	22.24	19.79	18.44	13.00	10.85	9.78	9.10	7.10	6.10
Soybean groats	18.50	18.50	18.50	17.00	17.00	17.00	15.40	15.40	15.40
Feeding yeast	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00	2.00
L-Lysine (78%)	0.10	0.10	0.10	0.20	0.20	0.20	0.23	0.23	0.23
DL-Methionine (99%)	0.22	0.22	0.22	0.30	0.30	0.30	0.30	0.30	0.30
Limestone	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10	1.10
Monocalcium phosphate	1.30	1.30	1.30	1.20	1.20	1.20	1.20	1.20	1.20
Salt	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35	0.35
Calcium formiate (30.5%)	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40	0.40
Captex T	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30	0.30
Premix	1	1	1	1	1	1	1	1	1
Protease	0.00	0.20	0.30	0.00	0.20	0.30	0.00	0.20	0.30

[1] Treatments: C-control group, standard broiler diet, without protease; E-I- broilers fed a diet with a 4% reduction in crude protein level as compared to the control group, and 0.2% protease supplementation; E-II broilers fed a diet with a 6% reduction in crude protein level as compared to the control group, and 0.3% protease supplementation.

On day 49 of the fattening trial, 10 male and 10 female broilers were randomly selected from each group, tagged, weighed and slaughtered after a fasting period of 10 hours.

At slaughter, conventionally dressed, ready-to-roast and ready-to-grill carcass weights and abdominal fat weight were determined and used to calculate dressing percentages and pre-slaughter abdominal fat percentage.

The data were subjected to conventional statistical methods. The significance of differences was tested by a two-factor analysis of variance using a 2x3 design (2 hybrids and 3 feeding treatments).

The significant differences determined by the analysis of variance and results of F-exp values were evaluated using Tukey's test. Significance was accepted at $P<0.05$. The tested parameters were subjected to an analysis of variance using ANOVA, *Microsoft STATISTICA Ver. 5.0, StatSoft Inc. (1995)*.

Results and Discussion

Table 2. presents conventionally dressed, ready-to-roast and ready-to-grill carcass weights and abdominal fat weight in broilers.

Table 2. Dressed carcass yield and abdominal fat weight of broilers (in g)

Hybrids	Groups		Pre-slaughter body weight	Conventionally dressed carcass weight	Ready-to-roast carcass weight	Ready-to-grill carcass weight	Abd. fat weight
Cobb 500	C (no protease)	$\bar{x}$	3181.0[a]	2753.4[a]	2596.9[a]	2378.9[a]	49.7[ab]
		Sd	318.4	271.6	246.8	234.6	12.7
	E-I (0.2% protease)	$\bar{x}$	3135.7[a]	2717.7[a]	2559.8[a]	2336.2[a]	53.6[a]
		Sd	291.2	233.9	213.6	203.6	15.4
	E-II (0.3% protease)	$\bar{x}$	3102.5[a]	2675.7[a]	2516.4[a]	2303.6[a]	48.7[ab]
		Sd	330.1	273.8	252.5	242.8	12.9
Master Gris	C (no protease)	$\bar{x}$	2570.2[b]	2200.5[b]	2056.0[b]	1834.6[b]	43.3[ab]
		Sd	192.7	165.9	151.4	136.8	13.5
	E-I (0.2% protease)	$\bar{x}$	2452.0[b]	2125.7[b]	1977.3[b]	1764.2[b]	40.8[b]
		Sd	210.2	184.1	163.2	148.0	11.4
	E-II (0.3% protease)	$\bar{x}$	2513.5[b]	2156.9[b]	2008.5[b]	1791.8[b]	46.1[ab]
		Sd	227.3	192.0	173.7	160.4	11.7
p-value							
Source of variation							
Hybrids			0.000	0.000	0.000	0.000	0.003
Protease			0.346	0.411	0.305	0.303	0.955
Hybrids x Protease			0.709	0.766	0.716	0.783	0.213

[a-b] Means with different superscripts within columns differ significantly (P<0.05)

At the end of the experiment, the fast-growing hybrid had a higher body weight than the slow-growing hybrid (P<0.05), which consequently had an effect on dressed carcass weights (conventionally dressed, ready-to-roast and ready-to-grill carcasses). Cobb 500 broilers had a somewhat higher abdominal fat weight compared to Master Gris broilers, with significant genotype-related differences occurring only between E-I groups. The different diet formulations had no significant effect on final body weight in either hybrid, or on carcass yield and abdominal fat content (P>0.05).

Given the above-mentioned results, dressed carcass yield was largely dependent on body weight at slaughter i.e. the average dressed carcass weights were higher in broilers that had higher average body weights at slaughter.

Data on dressing percentage and pre-slaughter abdominal fat percentage across the factors analyzed (hybrids, dietary treatments) are given in Table 3.

Table 3. Dressing percentage and abdominal fat percentage (in %)

Hybrids	Groups		Dressing percentage for conventionally dressed carcass	Dressing percentage for ready-to-roast carcass	Dressing percentage for ready-to-grill carcass	% abd. fat
Cobb 500	C (no protease)	$\bar{x}$	86.6	81.7[a]	74.8[a]	1.57
		Sd	0.91	1.08	0.91	0.40
	E-I (0.2% protease)	$\bar{x}$	86.7	81.7[a]	74.5[a]	1.72
		Sd	1.04	1.37	1.43	0.50
	E-II (0.2% protease)	$\bar{x}$	86.3	81.2[a]	74.3[a]	1.58
		Sd	0.86	1.03	0.84	0.43
Master Gris	C (no protease)	$\bar{x}$	85.6	80.0[b]	71.4[b]	1.71
		Sd	1.95	1.14	1.04	0.58
	E-I (0.2% protease)	$\bar{x}$	86.7	80.7[ab]	72.0[b]	1.68
		Sd	1.20	1.35	1.55	0.50
	E-II (0.2% protease)	$\bar{x}$	85.8	79.9[b]	71.3[b]	1.86
		Sd	1.25	1.27	1.37	0.54
p-value						
Source of variation						
Hybrids			0.041	0.000	0.000	0.166
Protease			0.038	0.067	0.209	0.751
Hybrids x Protease			0.277	0.489	0.313	0.346

[a-b] Means with different superscripts within columns differ significantly (P<0.05)

Table 3. shows that the broilers of both hybrids in all three experimental groups had a similar dressing percentage for conventionally dressed carcass and a similar abdominal fat percentage, with no differences as a result of tested factors observed (P>0.05). As regards the dressing percentage of ready-to-roast carcass, significance was found between C groups and E-II groups (P<0.05) (the fast-growing hybrid in these two groups had a higher dressing percentage compared to the slow-growing hybrid). Also, ready-to-grill carcass yield was higher in Cobb 500 than in Master Gris in all three groups (P<0.05), whereas no differences as a result of dietary treatments were observed (P>0.05).

Somewhat higher dressing percentages of Cobb 500 carcasses were obtained in this research compared to other studies *(Abudabos, 2010; Bogosavljević-Bošković et al., 2011; Nikolova et al., 2011)*. Master Gris broilers also had higher dressing percentages of conventionally dressed, ready-to-roast and ready-to-grill carcasses compared to those found for the same hybrid by *Blagojević et al. (2009)* at 91 days of age, *Škrbić et al. (2007)* for slow-growing Redbro broilers at 42 and 84 days of age, etc. The higher dressing percentages in this study are attributed to the higher

body weight of broilers at slaughter as well as to the longer duration of the grower stage (21 to 42 days) compared to other studies (grower stage up to 35 days).

The present results expectedly showed that fast-growing broilers had better dressing percentages compared to slow-growing birds, given their higher body weights and better body conformation scores. Similar results on better dressing percentages in fast-growing hybrids than in slow-growing broilers were obtained by *Grashorn and Clostermann (2002)* in their comparison between Ross strain (fast-growing hybrid) and ISA 657 genotype (slow-growing hybrid).

The feeding treatments (diet without protease and diets with crude protein levels reduced by 4 and 6 %, supplemented with 0.2 and 0.3% protease, respectively) had no effect on carcass quality parameters in broilers of either genotype, which is in compliance with the results reported by *Yadav and Sah (2005), Abudabos (2010)* and *Frietas et al. (2011).*

Abdominal fat percentage relative to body weight at slaughter was quite lower than in Cobb 500 broilers at 49 days of age in *Café et al. (2002), Ahmadi and Karimov (2010),* and in *Hajati et al. (2009)* – at 44 days of age.

Conclusion

The results suggest that the tested carcass quality parameters (weights and dressing percentages of conventionally dressed, ready-to-roast and ready-to-grill carcasses, and abdominal fat weight and percentage relative to body weight at slaughter) were significantly affected by genotype (the fast-growing hybrid had higher values for these traits compared to the slow-growing genotype). Moreover, the modifications of diet formulations (E-I and E-II groups fed diets with crude protein levels reduced by 4% and 6% through the use of reduced amounts of soybean meal, and supplemented with 0.2% and 0.3% Ronozyme Pro Act protease, respectively) had no significant effect on the tested carcass quality parameters in either hybrid compared to broilers given standard diets (C groups).

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Efekti proteaze dodate u hranu na masu i randman trupova tovnih pilića

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Rezime

U radu su prikazani rezultati istraživanja dodavanja enzima proteaze u hranu za piliće u tovu, uz snižen nivo sirovih proteina, na masu i udeo različito obrađenih trupova pilića dva tovna hibrida: brzorastućeg hibrida-Cobb 500 i spororastućeg hibrida-Master Gris. Potpune smeše za piliće iz oglednih grupa O-I i O-II imale su za 4, odnosno za 6% manje sirovih proteina u odnosu na kontrolnu grupu (C), s tim da je u njih dodat enzim proteaza (Ronozyme Pro Act) u koncentraciji 0,2, odnosno 0,3%. Na kraju ogleda 49.dana, slučajnim izborom, odabrano je iz svake ogledne grupe od oba hibrida po 10 muških i ženskih grla za klanje. Nakon klanja izmerena je masa klasično-obrađenog trupa, trupa „spremno za pečenje", trupa „spremno za roštilj" i abdominalne masti. Na osnovu ovih podataka i mase pilića pre klanja izračunati su randmani trupova i udeo abdominalne masti.

Rezultati istraživanja pokazali su da su ispitivane osobine kvaliteta trupova pilića bile pod značajnim uticajem genotipa, a da nije bilo efekta različitih hrambenih tretmana.

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EFFECTS OF DIFFERENT FORMS OF ZINC ON PLUMAGE CONDITION AND FOOT PAD LESIONS OF BROILER CHICKENS

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Original scientific paper

Abstract: The objective of the study was to investigate the effects of dietary organic and inorganic zinc on plumage condition and foot pad lesions of ROSS 308 broiler chickens. The experiment was carried out on 420 one-day old broilers randomly allotted to 2 groups with 6-replicates of 35-chickens each. First group was fed a standard corn-soybean diet supplemented with organic Zn (Vevomin Zn 13 % DSM - at 50% of the broiler strain recommendations) and second group inorganic Zinc sulphate (at 100% of the broiler strain recommendations). The plumage condition and severity of foot pad lesions were determined at the age of 35 days. Results of the trail showed that the plumage condition and foot pad lesions were not significantly ($P<0.05$) influenced by feeding different forms of zinc in the diet. Chickens fed standard corn-soybean diet supplemented with organic Zn did not have better plumage condition at 35 days of age compared with second inorganic group. It was found that broilers in both groups have problems with foot pad dermatitis without differences in foot pad lesions between the two observed groups.

Keywords: broiler, zinc, plumage condition, foot pad lesion

Introduction

Zinc is an essential inorganic trace mineral that is involved in a wide variety of physiological processes both for broiler health and growth. Zinc is involved in skin structure and protection *(McDowell, 2003)*. *Georgievski (1982)* reported that collagen synthesis, keratinization and cell replication require zinc as a cofactor, and its deficiency can lead to severe dermatitis, especially on the bird's footpad. *Sunde (1972)* reported that insufficiency of zinc in the diet of chicks caused dermatitis, especially of the feet, and poor feathering.

In many commercial poultry diets, the over supplementation of Zn, much higher than those recommended by *NRC (1994)*, was found to improve production

performance of broilers. A higher mineral supplementation though leads to wasteful and environmental contamination *(Bao et al., 2007)*. It has been suggested that the use of organic minerals at a much lower concentration for broiler diets counteracts this problem, because they have a higher bioavailability than inorganic salt analogues without any negative effect on production performance *(Midilli et al., 2014)* and potentially reducing mineral excretion *(Bao et al., 2007)*. *Zhao et al. (2010)* observed that 80 ppm of organic Zn in the diet improved footpad integrity and *Rossi et al. (2007)* also observed higher tissue resistance with the organic source.

Vieira et al. (2013) evaluated the efficacy of an organic source of dietary Zn compared to Zn sulphate, and concluded that organic Zn had no effect on humoral immune response, Zn concentration in the carcass, or production performance, but it reduced the incidence of footpad lesions. Improvement of skin quality and reduction of the severity and incidence of footpad lesions in broilers was observed when inorganic dietary Zn was partly replaced with organic Zn *(Saenmahayak et al., 2010)*. On the other hand, *Salim et al. (2012)* did not find any effect of diet supplementation with organic Zn on skin quality or on the growth performance of broiler chickens.

The objective of the study was to investigate the effects of dietary organic and inorganic zinc on plumage condition and foot pad lesions of ROSS 308 broiler chicks

Materials and Methods

The trial was carried out on the experimental farm of the Department of Animal Science of the Faculty of Agriculture in Novi Sad. A total of 420 one-day old broilers were randomly allotted to 2 groups with 6-replicates of 35-chicks each. First group fed standard corn-soybean diet supplemented with organic Zn (Vevomin Zn 13 % DSM - at 50% of the broiler strain recommendations) and second group inorganic Zinc sulphate (at 100% of the broiler strain recommendations).

Feed and water were offered *ad libitum* to animal throughout the trial. Broilers were fed with starter diet between 0-3 weeks, with grower diet between 3-5 weeks and a finisher diet in the last week of the trial. The starter included 22.00% crude protein, ME MJ/kg 12.60, grower diets 19.00% crude protein, ME MJ/kg 12.85 and finisher diet 17.00% crude protein, ME MJ/kg 13.00. Basic environmental parameters as well as the lighting program were in accordance to the technological demands of used hybrids..

In the 5^{th} week of age the feather condition was investigated on 72 broilers per group. The degree of feather damage was quantified in the six regions: head,

neck, breast, wings, back, and tail using a scale from 1 (poorly feathered) to 4 (fully feathered), according to the methods described by *Tauson et al. (1984)* and *Keppler et al. (2001)*. "4" being fully feathered without disorder, and "1" being significantly affected by the disorder. The six regions for plumage condition were summarized and indicated a total score ranging from 6 to 24 points. The foot pad lesions were evaluated at the end of the 5th week on 72 birds from each treatment and was calculated. Lesions were scored for both the left and right foot and classified according to a scale from 0 to 3, where zero represents no lesion and three the highest severity of damage *(Martrenchar et al., 2007)*.

Data were analyzed by ANOVA and means were separated by Duncan's post hoc test using StatSoft computer package (*STATISTICA 11, 2012*).

Results and Discussion

The results of the investigation of the total plumage condition and plumage scoring of the different body regions are presented in table 1. Plumage conditions shown were not significantly ($P<0.05$) influenced by feeding different forms of zinc in the diet. Chicks fed a standard corn-soybean diet supplemented with organic Zn did not have better plumage condition at 35 days of age compared with the second inorganic group. Based on average values of the evaluation of the total plumage condition, incomplete feathering in both chicken groups was established. The lowest feathering was observed in both groups on the breast. The broilers in both groups showed the best scoring on the wings.

Table 1. Average value with standard deviation for the total plumage condition and plumage scoring of the different body regions of broilers at 5 weeks of age

	Different sources of Zinc (Zn)			
	Inorganic		**Organic**	
	Mean	**SD**	**Mean**	**SD**
Head	2.10	0.97	2.18	1.06
Neck	2.29	0.88	2.42	0.97
Back	2.68	0.92	2.69	0.98
Breast	1.69	0.67	1.49	0.75
Wings	3.06	0.94	2.83	0.99
Tail	2.60	1.01	2.43	1.06
Total plumage condition	14.44	0.96	14.05	0.85

The foot pad lesions were evaluated at the end of the 5th week and are presented in table 2. We found that incidences of foot pad lesion was higher in broilers fed standard corn-soybean diet supplemented with inorganic Zn in

comparison with broilers from the second inorganic group. However, there was no significant difference ($p>0.05$) in foot pad lesions of the average score between two groups. Foot pads with no damage were found in both involved groups and there was no significant difference. More superficial lesions of the foot pads were found in the Organic Zn group (degree 1). The frequency of degree 2 was higher in the broilers fed diet supplemented with inorganic Zn. Also more deep epithelial lesions of foot pad (degree 3) were found in broilers fed standard corn-soybean diet supplemented with inorganic Zn (2.78%).

Table 2. Effects of dietary organic and inorganic zinc on foot pad lesions

					Frequency, %		
	Average Score	**SD**	**n**	**0**	**1**	**2**	**3**
Inorganic Zn	1.79	0.53	72.00	2.78	18.06	76.39	2.78
Organic Zn	1.69	0.57	72.00	2.78	29.17	66.67	1.39

The authors *Zhao et al. (2010), Rossi et al. (2007), Vieira et al. (2013), Saenmahayak et al. (2010),* concluded that diet supplementation with organic Zn improved overall skin quality and footpad integrity in broilers. This has not been confirmed in our research. Our results support the findings of *Salim et al. (2012)* who also found no effect of diet supplementation with organic Zn on skin quality broiler chickens.

Conclusion

Generally it can be concluded that plumage condition and foot pad lesions were not significantly ($P<0.05$) influenced by feeding different forms of zinc in the diet. Chicks fed a standard corn-soybean diet supplemented with organic Zn did not have better plumage condition at 35 days of age compared with second inorganic group. It was found that broilers in both groups have problems with foot pad dermatitis without differences in foot pad lesions between observed two groups.

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Uticaj raznih izvora cinka na stanje perja i lezija na nožnim jastučićima kod brojlerskih pilića

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Rezime

Cilj istraživanja je bio da se ispita uticaj organskog i neorganskog cinka na stanje perja i lezija na nožnim jastučićima kod Ross 308 brojlera. Eksperiment je izveden na 420 jednodnevnih pilića nasumično podeljenih na 2 grupe sa 6-ponavljanja i 35-pilića u svakom ponavljanju. Prva grupa je hranjena sa standardnom kukuruz soja smešom sa dodatkom organskog Zn (Zn Vevomin 13% DSM - 50% od preporuke za brojlere) u drugoj grupi je umesto organskog dodat neorganski cink-sulfat (100% od preporuke za brojlere). Ocena stanja perja i lezija na nožnim jastučićima je izvršena u uzrastu od 35 dana.

Rezultati su pokazali da perje stanje i i lezija na nožnim jastučićima nisu se statistički značajno razlikovali ($p < 0.05$) kod pilića hranjenih različitim izvorima cinka. Pilići hranjeni sa standardnom kukuruz-soja smešom sa dodatkom organskog Zn nije pokazala bolje stanje perje 35 dana starosti u poređenju sa drugom grupom sa dodatkom neorganskog Zn. Utvrđeno je su u obe grupe imale problema sa pojavom lezija na nožnim jastučićima.

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GROWTH PERFORMANCE AND BLOOD BIOCHEMICAL PARAMETERES OF BROILER CHICKENS FED DIET INCLUDED MEDICAL HERBS MIXTURE

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Abstract: Aiming to investigate the effect of medical herbs mixture (known on the market as FITOKOKCI-STOP) on productive performances and blood biochemical status, biological test were carried out on 80 one-day old Hubbard broilers. Broilers were divided into two dietary treatments within four replicates each. For the nutrition of chickens from control treatment (CON) basic commercial diet mixture was used, while the experimental treatment (MHM) was fed with the same basic mixture only with addition of 2.0 g/100g of a FITOKOKCI-STOP. From the obtained results it was concluded that addition of new product has led to the increase in body weight of chickens at the end of the sixth week of the experiment compared to the control treatment, without statistically significant differences ($P < 0.05$). Feed conversion ratio was uniform for the entire trial period and ranged between 2.05 and 2.19 kg of feed per kg of gain, without significant differences ($P > 0.05$). European broiler index was significantly higher in MHM treatment (226.4±4.21%) compared to control treatment (214.5±16.22%), while the mortality rate was significantly lower in MHM treatment (2.6±0.7%) in comparison to CON (5.2±0.8%). Significant differences were absent ($P > 0.05$) regarding the triglycerides and total cholesterol amounts. Furthermore, the low density lipoprotein (LDL) and non-high density lipoprotein (non HDL) in MHM treatment were significantly different compared to the CON treatment. From reported findings, it can be concluded that the addition of new product FITOKOKCI-STOP in broilers nutrition has positive influence on production performance and blood biochemical parameters of chickens.

Key words: medicinal herbs mixture, chickens, cholesterol, lipids, nutrition

Introduction

The antibiotic growth promoters (AGP) were banned in the European Union since 2006 due to the appearance of drug resistance in bacteria and the drug residues in meat *(Brenes and Roura, 2010)*. Nowadays, antibiotic resistant strains of bacteria have increased the concern about the potential public health problems and food safety, but feed too, is more seriously considered than before *(Sharifi et al., 2013)*. Striving to find alternative feed supplements considerable attention has been given to the medical herbs as replacements for AGP *(Ocak et al., 2008)*.

There is evidence suggesting that herbs, spices, and various plant extracts have received widespread attention worldwide and numerous reports exist in the literature *(Acamovic and Broker, 2005; Griggs and Jacob, 2005)*. Moreover, it is reported they have appetizing, digestion-stimulating and antimicrobial properties *(Sharifi et al., 2013)*. Furthermore, they act as antibacterial, antioxidant, anticarcinogenic, antifungal, analgesic, insecticidal, anti-inflammatory, anticoccidial agents, as well as immune-stimulants and growth promoters *(Tipu et al., 2006)*.

The effect of supplementation of dry peppermint (*Mentha piperita* L.) or thyme (*Thymus vulgaris* L.), which are among the alternative growth promoters, into animal feed on growth and blood profile of broilers has been reported by several authors *(Abdulkarimi et al., 2011; Feizi et al., 2013)*. *Artemisia absinthium* L., commonly known as wormwood belongs to the Asteraceae family and grows as a perennial herb with fibrous roots. The chemical constituents of leaves and flowers include silica, two bitter substances (absinthin and anabsinthine), thujone, tannic and resinous substances like malic and succinic acid *(Tariku et al., 2011)*. Wormwood is used as stomachic, antiseptic, antispasmodic, carminative, cholagogue, febrifuge and anthelmintic. The extracts of the plant have shown to exhibit strong antimicrobial and antioxidative activity *(Fiamegos et al., 2011)*. Moreover, *Artemisia absinthium* has been investigated as an alternative to antibiotics *(Brisibe et al., 2008; Kostadinović et al., 2015)*.

The aim of this study was to describe the effects of dietary inclusion of dry peppermint (*Mentha piperita*), wormwood (*Artemisia absinthium*), thyme (*Thymus vulgaris*) and wild thyme (*Thymus serpyllum*), which is new product called FITKOKCI-STOP, as growth promoter supplementation on growth performance and some biochemical blood parameters in broiler chickens.

Materials and methods

Animal housing

Experiments under *in vivo* conditions were carried out under production conditions at farm in Vojvodina, Serbia. At the beginning of the experiment, a total of 80 one-day old Hubbard broilers were distributed into two dietary treatments with four replicates each. One day old broilers were raised in a clean and disinfected room under standard conditions. For nutrition of chicks three mixtures were used, starter, grower and finisher through pan feeders (1/10 broilers). The first 14 days, during the preparatory period, chicks were fed with starter mixture. Following the preparation period, chicks were fed the next 21 days with grower mixtures, and then the last 7 days of fattening period with finisher mixtures according the experimental design given in Table 1. Broilers were with the access to water and feed *ad libitum.* Body weight was monitored at an individual level during the entire experimental period every seven days, while the feed consumption and feed conversion ratio were also monitored at pen level every seven days.

Table 1. Experimental design

Experimental treatments		Concentration of FITOKOKCI-STOP medicinal plants mixture in chicken diet		
		In starter (g/100g) 1-14 days	In grower (g/100g) 15-35 days	In finisher (g/100g) 36-42 days
CON	Control	0.0	0.0	0.0
MHM	Mixture of *Artemisia absinthium, Thymus vulgaris, Menthae piperitae, Thymus serpyllum* powder (1:1:1:1)	0.0	2.0	2.0

* Medical herbs mixture was added on top on the basic diet

European broiler index (EBI)

The European broiler index (EBI) was calculated for the entire feeding period according to the equation (*Koreleski et al., 2010*):

$$EBI = \frac{average\ body\ weight\ (kg) * survival\ rate\ (\%)}{age\ (days) * feed\ conversion\ ratio\ \ (kg\ feed\ /body\ weight\ gain)} * 100$$

Blood analyses

At the end of the 6th week, twelve birds were randomly chosen from each treatment and bled via wing vein puncture to obtain blood samples. Serum samples

from blood were separated by centrifugation (4000 rpm for 5 min at 20°C) *(Kostadinović, 1998)*. Commercially available kits (Randox Laboratories Limited – United Kingdom) were used to analyze the serum for triglycerides, total cholesterol, HDL and LDL on a biochemical auto analyzer Cobas Mira Plus (Roche Diagnostics). Values were expressed as mg/dl.

Data analyses

The results given in tables are reported as the mean ± standard deviations (SD). Significant effects were explored using analysis of variance (ANOVA) with repeated measurements, as well as Fisher's LSD post-hoc multiple range test to ascertain differences among treatment means. The data means were considered different at $P < 0.05$.

Results and Discussion

Based on the obtained results it can be concluded that the addition of medical plants mixture in the diet of broiler chickens led to significant ($P < 0.05$) differences in body weight (Table 2). Chickens have finished the preparatory period with uniform body weight with no significant differences ($P > 0.05$).

Table 2. Body weight of chickens (g)

	Treatments	
	CON	MHM
Initial mass	42.6±3.62^{a}	43.0±3.42^{a}
End of the 1st week	163.8±14.96^{a}	162.2±26.26^{a}
End of the 2nd week	385.2±46.68^{a}	385.6±70.11^{a}
End of the 3rd week	757.1±80.94^{a}	760.8±80.68^{a}
End of the 4th week	1222.6±170.15^{a}	1203.8±119.4^{b}
End of the 5th week	1617.6±144.17^{b}	1674.2±194.8^{a}
End of the 6th week	2059.8±210.63^{b}	2087.3±230.22^{a}

mean ± SD; n = 80

Treatments with different letter indexes in the same row are significantly different ($P < 0.05$)

At the end of the third week, chickens in treatment MHM have achieved slightly higher body weight (760.0 g) with no significant differences compared to treatment CON. However, after forth week control group showed slightly better results comparing to experimental treatment, but this difference was not statistically significant ($P > 0.05$). At the end of the second fattening period, addition of FITOKOKCI-STOP in treatments MHM exerted the stimulating effect and led to significant differences ($P < 0.05$) in body weight in relation to the control treatment. As the experiment closing to the end, it is notice that in MHM

treatment body weight of chickens was significantly higher ($P < 0.05$) compared to control treatment. At the end of the trial, body weight of MHM chickens was 2087.3 g, which is statistically significant difference ($P < 0.05$) compared to CON treatment (2059.8 g). Investigation of *Sharifi et al. (2013)* showed that the dietary addition of peppermint powder (15 g/kg) to broilers led to increased final body weights, which is also in agreement with the findings of *Ocak et al. (2008)* who concluded that powdered peppermint and thyme at 0.2% level may be incorporated as a growth promoter in the ration of broiler chicken. *Mehdipour et al. (2014)* showed that addition of *Thymus vulgaris* to Japanese quails nutrition has some positive effects on performance but none of them were significant. *Kostadinović et al. (2015)* investigated the effects of *Artemisia absinthium* supplementation in broiler diet on growth parameters and have noticed that significantly higher live weight ($P < 0.01$) was observed in the broilers treated with 200 g/kg *Artemisia* whole plant at 6 weeks of age compared to other treatments. Furthermore, during the trial experimental groups had better feed conversion efficiency.

From the results given in Table 3 it can be noticed that feed conversion ratio in preparation period of chickens was uniform and ranged between 1.38 and 1.39 kg of feed per kg of gain, without significant ($P > 0.05$) differences. In the grower phase the lower achieved feed conversion ratio was in treatment CON (1.83 kg/kg), while in group MHM this value was slightly higher (1.94 kg/kg). Feed conversion ratio in finisher phase was significantly higher ($P < 0.05$) in experimental treatment MHM (3.25 kg/kg) in comparison to control treatment (2.94 kg/kg). *Feizi et al. (2013)* showed that the addition of thyme volatile oils to broilers nutrition significantly improved feed conversion ratio which clarified that the broilers fed rations supplemented with thyme utilized their feed more efficiently than those fed rations without addition of this herb. *Mehdipour et al. (2014)* concluded that addition of thyme has no effect on feed conversion ratio, which is not in accordance with results obtained in our study.

Table 3. Feed Conversion Ratio (kg/kg)

	Treatments	
	CON	MHM
Starter 1-14 days	1.39 ± 0.00^{a}	1.38 ± 0.00^{a}
Grover 15-35 days	1.83 ± 0.05^{a}	1.94 ± 0.07^{a}
Finisher 36-42 days	2.94 ± 0.23^{a}	3.25 ± 0.02^{a}
Entire period 1-42 days	2.05 ± 0.79^{b}	2.19 ± 0.95^{a}

mean ± SD; n = 80
Treatments with different letter indexes in the same row are significantly different ($P < 0.05$)

Results regarding the European broiler index (EBI) and chicken mortality rate are presented in Table 4. Addition of medical herbs mixture led to a significant

(P < 0.05) increase in values of EBI of the experimental treatment (226.43%) in comparison to the control treatment (214.5%). In the control treatment was recorded significantly higher (P < 0.05) mortality rate of chickens compared to experimental treatment. Positive results concerning EBI points were recorded also in research of *Arczewska-Wlosek and Swiatkiewicz (2012)* with addition of blend extract containing thyme to chicken diets. Investigation of *Puvača et al. (2015)* also showed that addition of different phytoadditives also has impact on European broiler index.

Table 4. European broiler index and mortality

	Treatments	
	CON	MHM
EBI	214.5±16.22[b]	226.43±4.21[a]
Mortality	5.2±0.8[a]	2.6±0.7[b]

mean ± SD; n = 80
Treatments with different letter indexes in the same row are significantly different (P < 0.05)

FITOKOKCI-STOP powder supplementation in broiler diet indicates high improvement of blood lipid profile. Results given in Table 5 showed that higher amounts of triglycerides (63.2 mg/dl), total cholesterol (98.6 mg/dl) and LDL (38.4 mg/dl) were in control treatment with significant (P < 0.05) differences in comparison to the MHM treatment. Addition of FITOKOKCI-STOP in the amount of 2.0 g/100 g (P < 0.05) decreased the concentration of triglycerides (61.7 mg/dl) in blood serum. This effect can be explained by the possible inhibition of the Acetyl CoA syntheses enzyme that is necessary for the biosynthesis of fatty acids. FITOKOKCI-STOP supplementation decreased LDL level compared to the levels in chickens of the control treatment. This effect can be explained by the possible mechanism of antioxidant and antiperoxide lowering action on LDL or the decrease in hepatic production of very low density lipoprotein (VLDL) which serves as the precursor of LDL in the blood circulation (*Puvača et al., 2015*). In investigation of *Abdulkarimi et al. (2011)* there was no significant difference between the treatments for plasma triglycerides, but plasma total cholesterol and LDL concentration of thyme extract received birds were lower (P < 0.05) compared with those of control chickens, which is in accordance to results obtained in this study. On the other hand, *Fallah et al. (2013)* came to the statement that addition of *Mentha pipperita* in broiler nutrition have no effect on HDL and LDL concentration, which is in contrary to our results.

Table 5. Biochemical blood parameters and lipid profile (mg/dl)

	Treatments	
	CON	MHM
Triglycerides	63.2±0.21[a]	61.7±1.23[a]
Total cholesterol	98.6±0.98[a]	96.5±0.47[a]
HDL	17.9±1.47[b]	42.8±1.28[a]
LDL	38.4±2.04[a]	2.7±1.76[b]
Non HDL	80.7±0.55[a]	53.7±0.41[b]
HDL/LDL	0.47±1.75[b]	15.85±1.52[a]

mean ± SD; n = 12
Treatments with different letter indexes in the same row are significantly different ($P < 0.05$)

The higher concentration of HDL (42.8 mg/dl) was recorded in MHM treatment. Furthermore, results of this study indicate the significant ($P < 0.05$) increase of HDL by addition of medical herbs mixture (42.8 mg/dl) compared to CON treatment (17.9 mg/dl).

Conclusion

Based on the obtained results, it can be concluded that the addition of FITOKOKCI-STOP medical plants mixture in broiler chicken nutrition has positive effect on production performances and blood lipid profile. Addition of FITOKOKCI-STOP in the amount of 2.0 g/100g has led to the higher final body weights compared to control group. Moreover, the feed conversion ratio and mortality were significantly improved in experimental group compared to control chickens. Also it can be concluded that significant lowering of plasma cholesterol, triglycerides, LDL and increase of HDL by FITOKOKCI-STOP powder supplementation in broiler diet indicates effective results in regulation of lipid metabolism in a favorable manner for prevention of atherosclerosis or coronary heart diseases in humans who use this kind of chicken products in their daily nutrition.

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Uticaj dodatka smeše lekovitog bilja na proizvodne performanse i biohemijske parametre krvi brojlera

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Rezime

U cilju ispitivanja uticaja smeše lekovitog bilja (na tržištu poznata kao FITOKOKCI-STOP) na proizvodne performanse i biohemijske parametre krvi, za ogled je korišćeno 80 jednodnevnih Hubbard pilića. Pilići su podeljeni u dva tretmana, a svaki hranidbeni tretman imao je četiri ponavljanja.Kontrolne grupe pilića (CON) hranjene su komercijalnom hranom, dok su pilići iz eksperimentalnog tretmana (MHM) hranjeni istom hranom ali sa dodatkom FITOKOKCI-STOP-a u količini od 2.0 g/100 g. Na osnovu dobijenih rezultata ustanovljeno je da je dodatak novog proizvoda na bazi lekovitog bilja doveo do porasta u težini pilića na kraju šeste nedelje ogleda, u poređenju sa kontorlnim tretmanom, ali bez statistički značajne razlike ($P < 0.05$). Konverzija hrane je bila uniformna tokom celog ogleda i kretala se od 2.05 do 2.19 kg hrane/ kg prirasta, bez statistički značajne razlike ($P > 0.05$). Evropski brojler indeks je bio statistički značajno viši u MHM tretmanu (226.4±4.21%) u odnosu na kontrolni tretman (214.5±16.22%), dok je smrtnost pilića bila značajno niža u MHM tretmanu (2.6±0.7%) u poređenju sa CON grupama (5.2±0.8%). Značajna razlika između tretmana nije uočena ($P > 0.05$) kada je reč o trigliceridima i ukupnom holesterolu. Takođe, lipoproteini male gustine (LDL) and lipoprteini koji nisu velike gustine (non HDL) u MHM tretmanu su bili značajno različiti u poređenju sa CON tretmanom. Na osnovu dobijenih podataka, može se zaključiti da je dodatak novog proizvoda FITOKOKCI-STOP u ishrani pilića imao pozitivan uticaj na proizvodne performanse i biohemisjke parametre krvi brojlera.

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THE INFLUENCE OF EGGSHELL COLOR ON MEAT COMPOSITION OF PHEASANTS (Phasianus colchicus)

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Original scientific paper

Abstract: The aim of this paper was to determinant the influence of egg color on the meat composition of fully grown pheasants, at the age of 150 days. The highly valued meat portions from pheasants are breast and thigh muscles. The main parameters of meat quality are the percentage of dry mass and moisture and the percentage of total proteins and fat. Many factors influence these parameters and the eggshell color is one of them. The shell color of eggs laid by the pheasant female is preconditioned genetically. The color intensity decreases with the duration of the laying period and the following four basic pheasant eggshell colors can be distinguished: dark-brown, light-brown, olive and blue. Correlations have been observed between eggshell color and some egg physical traits as well as egg fertilisation and chicks hatching. Moreover, the course of embryogenesis and, consequently, hatching results can also be influenced by the egg color. Our data shows that the final product (meat) can also be affect by this factor. Meat of pheasants hatched from eggs of blue eggshell color has a significantly lower dry matter content and higher moisture content. The meat of these pheasants contains significantly less fat. The eggshell color of pheasants eggs has no influence on the content of total protein in meat. These results can be explained by the difference in the development of the muscle tissue and connective tissue within the muscle. This can be a important factor in the selection of pheasants eggs for incubation.

Key words: pheasants, eggshell color, meat composition

Introduction

Meat from pheasants is a highly valued food. Pheasants contribute substantially to the hunting bags in European countries, with significant benefits for local economy. Hunted pheasants are either wild-born feral or were bred in aviaries and then released. Farming of pheasants is done not only for restocking/release in hunting but also for slaughter and meat production *(Golze, 2010).*

The highly valued meat portions from pheasants are breast and thigh muscles. However meat quality has to be considered, because it will substantially influence the consumers attitude to select and buy such meat or products. The main parameters of meat quality are the percentage of dry mass and moisture and the percentage of total proteins and fat. Many factors influence these parameters and among them are genetic and paragenetic factors *(Frenco and Lorenzo, 2013).*

The shell color of eggs laid by the pheasant female is preconditioned genetically and the color intensity decreases with the duration of the laying period. The following four basic pheasant eggshell colors can be distinguished: dark-brown, light-brown, olive and blue *(Richards and Deeming, 2001)*. *Krystianiak and Kontecka (2002)* observed in some pheasant populations that 8, 20, 24 and 48% of females laid eggs with the following eggshell color: blue, olive, dark-brown and light-brown. However, the other authors found a few shades of mentioned eggshell colors *(Richards and Deeming, 2001; Kirikçi et al., 2005).*

There are few articles in literature on the subject concerning pheasant egg quality depending on the eggshell color *(Mróz and Pudyszak, 2000; Richards and Deeming, 2001; Kirikçi et al., 2005; Krystianiak et al., 2005)*. In addition, no data was found in the literature on the subject about the influence of egg color on the meat composition of the fully grown pheasants.

On the other hand, correlations have been observed between eggshell color and some egg physical traits *(Scott and Silversides, 2000; Krystianiak and Kontecka, 2002; Silversides and Budgell, 2004)* as well as egg fertilisation and chicks hatching *(Mróz and Pudyszak, 2000)*. Moreover, the course of embryogenesis and, consequently, hatching results can also be influenced by the egg color. These data suggest that the final product (meat) can also be affect by this factor.

The aim of our study was to determinant the influence of egg color on the meat composition of fully grown pheasants, at the age of 150 days. Profitability of pheasant rearing depends, primarily, on their productive possibilities *(Deeming and Wadland, 2001; Kirikçi et al., 2003)* and our result shows that meat quality can be enhanced by the selection of eggs based on their colors.

Materials and Methods

For this trial we have used the fertilized eggs of pheasants (Phasianus colchicus). After the acquisition, the eggs are divided into four experimental groups (Eg). The experimental groups were formed according to the eggshell colour: EgD – dark brown, EgL - light-brown, EgO – olive and EgB – blue.

The eggs, from each experimental group, were placed in a individual incubator, where they are incubated for up to 25 days. After 25 days the birds were

labeled (with leg rings) and transferred to a facility for breeding until the age of 6 weeks with conventional floor housing system. The temperature in the house on the day of admission was 35°C, and after entering, the temperature was decreased by 1°C each day, until reaching the outside temperature. At the age of 6 weeks the birds were transferred to cages where they were held until the age of 150 days. During the experiment, pheasants were fed *ad libitum* and were under constant veterinary supervision.

At the age of 150 days, from each experimental group were taken 10 birds and sacrificed. The samples of breast musculature (*m. pectoralis superficialis*) were taken from each individual bird to determinate the composition of meat. The following parameters were determined: the content of dry matter and moisture and total protein content and total lipids in meat and in dry matter.

The moisture content in meat samples was determined by drying at 105˚C to constant weight. The percentage of protein in meat was determined by Kjeldahl method. The percentage of fat in meat was determined by Soxlet method.

The results were presented as g/100g ± standard deviation. The degree of statistical significance between groups was performed by analysis of variance (ANOVA) and post hock Tukey´s tests. Statistical tests were carried out using the software package Statistica for Windows, ver. 10.0 (StatSoft, Tulsa, OK, USA).

Results and Discussion

Results obtained by chemical analysis of meat are shown in Table 1. The share of dry matter and moisture differed significantly ($p<0,05$) between the experimental group hatched from blue eggs compared to all other experimental groups. Also, in this experimental group, lipid content was significantly ($p<0,05$) lower compared to the other experimental groups. There was no significant difference in total protein content between all experimental groups.

Table 1. Meat composition

Parameter	Experimental group			
	D	L	O	B
Dry matter	30,74±1,31[a]	31,54±0,91[a]	30,67±0,85[a]	29,66±1,32[b]
Moisture	69,26±1,31[b]	68,46±0,91[b]	69,33±0,85[b]	70,34±1,32[a]
Total protein	26,27±0,67	26,38±0,99	26,46±1,11	26,11±1,10
Total fat	0,88±0,16[a]	0,73±0,18[a]	0,61±0,11[a]	0,22±0,12[b]
Total protein in dry matter	85,55±2,89	85,08±3,93	86,30±3,61	86,98±4,60
Total fat in dry matter	2,81±0,11[a]	2,38±0,09[a]	1,90±0,13[a]	0,77±0,17[b]

[a, b] Means within a row with no common superscript differ significantly ($P < 0.05$).

Experimental group B had significantly lower dry matter content, which ranged below 30 g/100g, and significantly higher moisture content above 70g/ 100g. This ratio indicates a lower content of nutritionally most valuable components of meat, primarily proteins and lipids. In addition, the meat with a higher content of moisture has a potentially greater possibility of the loss of water upon its cooling down to + 4° C after a 24 h and 48 h (drip loss), and thus the possibility of reducing the weight of the final product. However, the percentage of dry matter and moisture in meat is not sufficient indicator of the quality of meat and its organoleptic properties.

Meat of pheasant, particularly breast and thigh muscles, contains a high percentage of protein, which is significantly higher than in domestic poultry. This protein content gives the high nutritional value to pheasants meat. Data obtained in this study indicate that the eggshell color do not affect significantly the protein content in raw meat, as well as in dry matter. In all experimental groups, the protein content in raw meat ranged above 26 g/100g, while the total protein content in the dry matter ranged above 85 g/100g in the experimental groups D and L, and above 86 g/100g in the experimental groups A and B.

The results indicate that meat from pheasants, hatched from egg with blue eggshell color, contains significantly less fat content, both in raw meat and in dry matter. Among other experimental groups there was no statistically significant difference in the fat content in raw meat and in dry matter. These results indicate that a higher percentage of dry matter, in these experimental groups, is a consequence of increased fat content. A higher proportion of fat in these groups indicates better organoleptic characteristics of the meat.

These results can be explained by the difference in the development of the muscle tissue and connective tissue within the muscle *(Uśćebrka et al., 2014)*. Muscles, from pheasants that are hatched from blue eggshell color eggs, have more cells with smaller diameter in the same area compared with the pheasants that are hatched from the eggs of other colors. In contrast to the diameter and the number of muscle cells, there was no statistically significant difference in the prevalence of connective tissue within the muscle, at a pheasant hatched from eggs of different colors *(Žikić et al., 2014)*. This can be consequence of the thinner and more porous eggshell of blue eggs, wich leads to greater water loss during the incubation *(Kijowski and Rosinski, 2009; Kozuszek et al., 2009)*. This can be related to lower viability of chickens hatched from these eggs and consequentialy the weaker development of tissues and organs, such as muscle tissue (*Krystianiak et al., 2005).*

This indicates that the weaker development of muscle tissue of pheasants hatched from eggs of blue eggshell color can influence the composition and quality of meat.

Conclusion

The eggshell color of pheasants eggs significantly influence the composition and quality of meat at the age of 150 days. Meat of pheasants hatched from eggs of blue eggshell color has a significantly lower dry matter content and higher moisture content. The meat of these pheasants contains significantly less fat. The eggshell color of pheasants eggs has no influence on the content of total protein in meat. This can be a significant factor in the selection of pheasants eggs for incubation.

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Uticaj boje jaja na sastav mesa fazana (Phasianus colchicus)

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Rezime

Cilj ovog rada je bio da se utvrdi uticaj boje jaja na hemijski sastav mesa potpuno odraslih fazana, u uzrastu od 150 dana. U visoko cenjeno meso fazana se naročito ubraja grudna muskulatura i muskulatura bataka. Osnovni parametri kvaliteta mesa fazana su procenat suve mase i vlage, kao i procenat ukupnih proteina i masti. Mnogi faktori utiču na ove parametre a boja ljuske jaja je jedan od njih. Boja ljuske jaja koje legu ženke fazana ima svoju genetski utvrđenu osnovu. Intenzitet ove boje se smanjuje sa trajanjem perioda nošenja i sledeće četiri osnovne boje ljuske jaja fazana se mogu razlikovati: tamno-braon, svetlo-braon, maslinasta i plava. Korelacija između boje ljuske i pojedinih fizičkih osobina jaja je dokazana. Pored ovoga, utvrđena je i veza između stepena oplodnje jaja i procenta leženosti. Osim toga tok embriogeneze i, samim tim, sam proces leženja može biti pod uticajem boje jaja. Naši podaci ukazuju na to da je finalni proizvod (meso), takođe, pod uticajem ovog faktora. Meso fazana izleženih iz jaja plave boje ljuske ima statistički značajno niži sadržaj suve materije i veći sadržaj vlage u odnosu na fazane izležene iz jaja drugih boja. Takođe, meso ovih fazana je sadržalo statistički značajno manje masti, u ukupnoj masi i u suvoj materiji. Boja ljuske jajeta fazana nije imala uticaja na sadržaj ukupnih proteina u mesu. Ovi

rezultati mogu se objasniti razlikom u razvoju mišićnog tkiva, kao i zastupljenosti vezivnog tkiva unutar samog mišića. Ovaj uticaj može biti vrlo važan faktor prilikom izbora jaja fazana za inkubaciju.

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EVALUATION OF ADAPTIVE CAPACITY OF KIDS ON THE BASIS OF REALIZATION SPEED OF BEHAVIOURAL REACTIONS DURING THE FIRST HOUR AFTER BIRTH

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Abstract: The development in the behaviour of the kid, within the first hours after its birth, provides information for its adaptive capacity and the necessity to optimize and adapt the breeding technologies in order to speed the formation of the relationship between goat-mother and kid, the protection of new-borns and the increase in reproductive efficiency. The behaviour of 32 kids were observed from Bulgarian White Milk (BWM) breed, 13 kids from BWM x Anglo-Nubian (AN) and 15 kids from BWM x Togenburg (TG) of different sex and type of delivery. The single kids made their first attempt to rise to their feet averagely on 6.0±1.2 min, and they Successfuly rose stood on their feet on 24.9±2.9 min., which was shorter than the time till their first attempt for rising and the successful standing for the twins – respectively 7.9±1.3 min. and 28.3±2 min. The single kids spent almost two minutes more on their feet (P>0.05) than twin kids (22.6±2.9 min. against 20.9±2.3 min.). Male and female kids made their first attempt to rise almost at one and the same time (female 6.8±1.2 min and male 6.6±1.1 min.) after their birth. The time till the first successful attempt to rise up was influenced reliably by the sex of the new-born – female kids rose up earlier than male averagely with 6 min (on 29.8±2.5 min for male and on 23.8±1.6 min for female). The female kids stood longer time on their feet within the first hour after birth (22.7±2.6 min), but the difference in comparison with the male ones (20.1±2.4 min) was not proven. The single kids made their first attempt to suck on 25.7±2.8 min and they sucked successfully averagely on 33.0±4.2 min after their birth, as it was earlier in comparison with twins, respectively on 31.8±2.5 and 40.3±3.2 min. Among the kids that were observed, it was noticed that female kids made their first attempt to suck earlier (28.6±2.9 min.), but they started sucking later (39.3±3.4 min.) and they spent shorter time in sucking during the first hour after their birth (6.3±1.8 min.) in comparison with the male kids (31.3±2.5 min.; 34.4±3.5 min. и 7.6±2.5 min.).
The aim of the present study was to follow the expression of behaviour of kids during the early neonatal period.

Key words: kids, behaviour, sucking

Introduction

The new-born kids have a high degree of independence and are able to rise to their feet and start sucking soon after their birth. The behavioural model of the kid is oriented towards the establishment of a connection with the mother and starting sucking in the sensitive period after birth *(Ramirez et al., 1998)*.

The time necessary for the new-born to rise and find the udder is of utmost importance in order to start sucking on time. Neonatal death is the highest during the first three days, which presumes, that the events occurring within that period are of crucial significance for the survival *(Nowak et al., 2000)*. The manifestation of behaviour of lamb after birth is influenced by factors arising from the kid itself (breed, line, sex etc.), prenatal influences (feeding, number of new-borns), factors arising from the sheep (the size of placenta, body condition and age of the mother) and the course of the birth process *(Dwyer, 2003)*. The behaviour of kids immediately after their birth is oriented towards finding the udder and starting sucking. The first movements are getting up and shaking of the head, followed by falling, bleating, kneeling, and after that attempts for rising, first standing on their hind limbs, followed by stretching of the forelimbs. These behaviours could be fulfilled quickly within a few minutes after birth, but most lamb rise up in the course of 30 minutes after birth *(Dwyer, 2003)*. In its attempt to start sucking, first the kid turns to the forelimbs of the mother-goat, then it finds its way to the hind limbs and the inguinal region, although it has not had a contact with the udder yet, it could make suctorial movements *(Ramirez et al., 1998). O'Brien and Sherman (1993)* found that new-born kids, as well as other new-borns from other livestock species, with syndesmochorial or epitheliochorial placentation, depend on the colostrum that they receive. Through it, they take up antibodies and build passive immunity till the moment, when they start producing antibodies by themselves. The inability to take up colostrum timely could increase the incidence of infectious diseases and death among new-born kids. It was found that the normal sucking and assimilation of colostrum is a vital factor crucial for health *(Khan et al., 2006)* and the normal growth during the neonatal development, and it is also more important than birth weight *(Chen et al., 1999)*.

The aim of present study is to assess the adaptive capacity of kids on the basis of speed of realization of behavioural reactions during the first hour after birth.

Material and Methods

The experiment was conducted in the period from 2011 till 2014, at the Experimental Base at RIMSA – State Enterprise, Troyan. The behaviour of 32 kids were studied from Bulgarian White Milk (BWM) breed, 13 kids from BWM x AN and 15 kids from BWM x TG of different sex and type of delivery. Chronometers were used, in the course of observation of the animals, to give an account of the time of all variables.Kids were observed by two researchers and the observation lasted for one hour after birth.

The following behavioural reactions, registered within the first hour after birth, were assessed: Time till the first attempt for rising - duration of the period from birth (pushing out) till the first attempt of the kids to stand up. Time till the first rising - duration of the period from birth till the first successful attempt for rising (the kid remains standing on its 4 limbs at least for 5 seconds).

Total time of standing - time during which the kid remains standing on its feet within the first hour of the postnatal life. Time till the first attempt for sucking - time from birth till the first attempt for sucking. Time till the first successful sucking - time from birth till beginning of sucking. Total time for sucking - time spend in sucking in the course of the first hour after birth. Data are presented as mean value ($\bar{x}$) and error of the mean ($S\bar{x}$). Results from all tasks were processed with statistical tools package program Windows (Microsoft Excel, 2003), and the significance was calculated by ANOVA method through single-factor and two-factor analysis.

Results and Discussion

The effect of type of birth and sex of the kid on time for rising within the first hour after birth

The single kids made their first attempt to rise averagely on 6.0±1.2 min and successfully rose on their limbs on 24.9±2.9 min, which was shorter than time till the first attempt to rise and the successful standing up for the twins – respectively 7.9±1.3 min and 28.3±2.0 min (Figure 1). The single kids spend almost two minutes more standing on their limbs ($P>0.05$) than twin kids (22.6±2.9 min compared to 20.9±2.3 min).

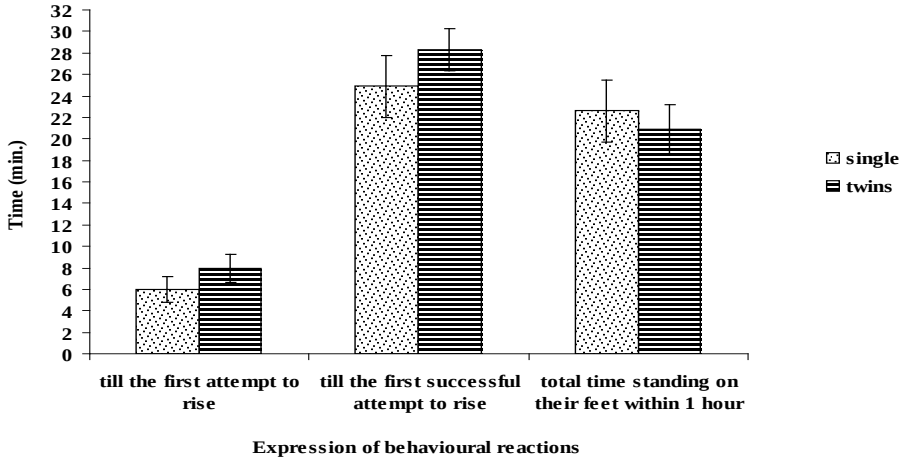

Fig. 1. The effect of type of birth on time till the rising of the kid within the first hour after birth

Awotwi et al. (1999) report that single kids from West African Dwarf goat breed stand steadily on their feet for 12.9±4.5 min after birth, and twins for 13.8±7.3 min. This time is shorter in comparison with that we found, but as in our study the single kids rose up more quickly in comparison with twins. The degree of maternal care has no significant effect on the time till the first rising, but it has an effect on time till the first attempt for sucking and the time till the start of sucking *(Martinez et al., 2009)*. For some mothers the decreased motor activity in kids could stimulate increased attention towards new-borns. There is a positive correlation between time till the first attempt for sucking and time till the first sucking. The behaviour of kids within first hour after birth is related with live weight at birth. For kids with higher birth weight, birth was more prolonged, which lengthened time from rising to the start of sucking - (37.32 min for single and 25.69 min for twin kids), but they compensated this delay, as at the end of the first hour the total time spent in sucking was more prolonged than for the twins (5.86 min in comparison with 2.99 min). These results are similar to those of *Ramirez et al. (1998*) for goats with two and more pregnancies. Time till the first attempt for rising corresponded with the type of birth and it was shorter for single than twin kids (7.18 min in comparison with 8.67 min). It is supposed that the difference was a result of the higher level of maternal care, as the attention of the mother did not divert towards the next birth. The authors did not register reliable differences between male and female kids in relation to time till the first attempt for rising. The effect of sex on the expression of behavioural reactions in our study is presented in Figure 2. Male and female kids made their first attempt to rise almost at the same

time (female 6.8±1.2 min and male 6.6±1.1 min) after birth. The time till the first successful attempt for rising was influenced significantly by the sex of the new-born – female kids rose up earlier than male averagely with 6 min (on 29.8±2.5 min for male and on 23.8±1.6 min for female). The female kids stood longer time on their feet within the first hour after birth (22.7±2.6 min), but the difference in comparison with male ones (20.1±2.4 min) was not proven.

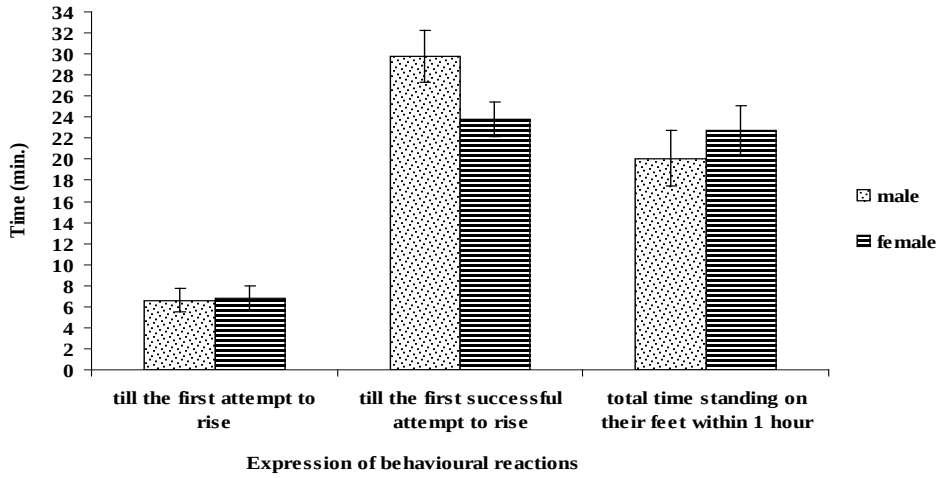

Fig. 2. The effect of sex on time till rising of the kid within first hour after birth

There is not enough information for the existence of genetically determined differences in the stage of physiological maturity at the moment of birth, which determines the differences in the physical activity of new-borns of different sex and type of birth. The amount of energetic reserves of kids, having different weight at the moment of birth, would not have effect either on their activity within the first minutes of postnatal life, and respectively on time till the first successful attempt for rising of kids with lower weight. The shorter time for rising of female kids probably is connected with the lower weight and shorter duration of birth, as well as with the quality of maternal care, expressed in the longer time spent in care for them within the first hour after birth. The shorter time till the successful rising in our study could not be regarded as a prerequisite for increasing of the survival, because it did not lead to earlier sucking.

The effect of type of birth and sex of kid on time till the start of sucking within first hour after birth.

The single kids made their first attempt to suck averagely on 25.7±2.8 min and they sucked successfully averagely on 33.0±4.2 min after their birth (Figure 3), as it was earlier in comparison with twins, respectively on 31.8±2.5 and 40.3±3.2 min.

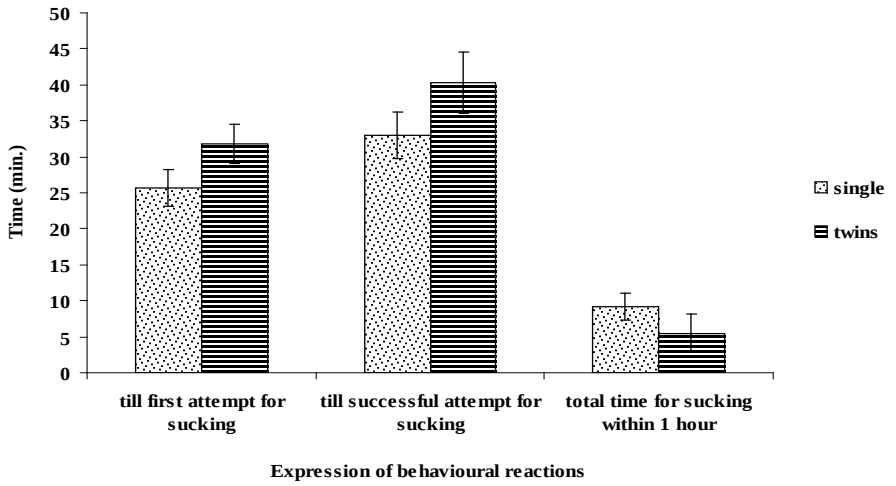

Fig. 3. The effect of birth type on time till the kid starts sucking

Awotwi et al. (1999) reported that the single kids start sucking more quickly than twins, respectively 14.3±6.6 min for singles and 15.7±6.2 for the first one of the twins. The time, they have registered, till the start of sucking of new-borns was considerably shorter than the time in our trial. According to the authors the earlier start of sucking of new-borns gives a better opportunity to take up more colostrum and respectively a greater amount of antibodies from the mother. In the study on goats of Murciano – Granadina breed, *Martinez et al. (2009)* found, that single kids made their first attempt to suck later than twins (34.76±10.26 min in comparison with 22.45±10.26 min), and the successful sucking was also implemented later in comparison with twins (37.32±6.73 min in comparison with 25.69±10.51 min). The differences between studies could be due to the differences among breeds studied. According to the indicator of total time for sucking within first hour after birth in our study, the single kids spent more time in sucking, in comparison with twin kids, as the difference in time was more than 3 minutes (9.2±2.6 min for singles and 5.5±1.9 min for twins). *Martinez et al. (2009)* reported that 70% of new-born single kids had made an attempt to start sucking within the first hour as only 41% of them succeeded to suck. For comparison, 94 % of new-born kids had made an attempt to start sucking, as 83% from them did it

successfully. They found that the type of delivery and duration of birth had an effect on the total time for sucking during the first hour after birth. These results correspond to those obtained in our study, but the values we registered for the controlled indicators were higher. *Ramírez et al. (1998)* report that 100% of kids delivered by goats, which had two and more kids, had made an attempt to suck, as 93% of single kids and 88% of twin kids started sucking successfully. One of the reasons for earlier and more continuous sucking for single kids is that they are more active and make more attempts for sucking in comparison with twin kids *(O'Connor et al., 1992)*. Among the kids that were observed, it was noticed (Figure 4), that female kids made their first attempt to suck earlier (28.6±2.9 min.), but they started sucking later (39.3±3.4 min.) and they spent shorter time in sucking during the first hour after their birth (6.3±1.8 min.) in comparison with male kids (31.3±2.5 min.; 34.4±3.5 min. and 7.6±2.5 min.).

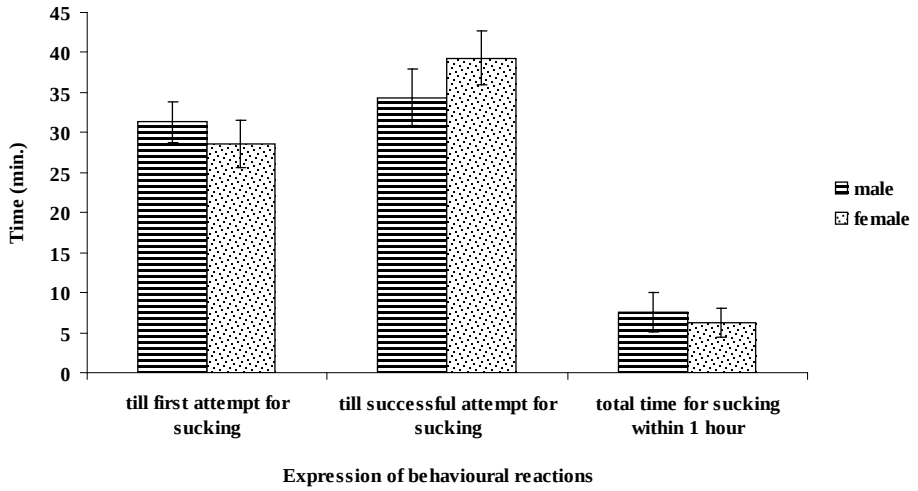

Fig. 4. The effect of sex on time till sucking within first hour after birth

Similar results were reported by *Martinez et al. (2009)*, who stated that female kids started sucking later and spent less time in sucking in comparison with male animas as the differences were not proven. The authors did not find significant differences in relation to total number of kids, which started sucking successfully within the first hour after birth, which constituted respectively 66% in male and 65% in female animals. 82% of male and 85% of female kids made attempt to start sucking, and 66% of male and 65% of female started sucking successfully.

Conclusion

The time till the first successful attempt to rise up was influenced significantly by the sex of the new-born – female kids rose up earlier than male averagely with 6 min.

Sex and type of birth did not had a significant effect on the total time for sucking in the course of the first hour after birth, which comprised respectively (7.6±2.5 min), (6.3±1.8 min), (22.6±2.9 min), (20.9±2.3 min) for the male and female kids, singles and twins.

Procena sposobnosti prilagođavanja jaradi na osnovu realizacije brzine reakcija u ponašanju tokom prvog sata nakon rođenja

S. Stoycheva, Ts. Hristova, P. Zunev, Y. Alexiev, Ts. Dimitrova

Rezime

Razvoj ponašanja jareta, u prvim satima posle njegovog rođenja, daje informacije za adaptivni kapacitet i neophodnosti da se optimizira i prilagodi tehnologija parenja kako bi se ubrzalo formiranje odnosa između koze-majke i jareta, zaštite novo-rodjena jarad i poveća reproduktivna efikasnost. Praćeno je ponašanje 32 jareta bugarske bele mlečne rase (BWM), 13 jaradi meleza BWM x anglo-nubijske rase (AN) i 15 jaradi meleza BWM x togenburška rasa (TG) različitog pola i jarenja. Jedinci su svoj prvi pokušaj da se uzdignu na nogama u proseku imali posle 6,0 ± 1,2 minuta, a uspešno su ustajali i stajali na nogama posle 24,9 ± 2,9 min., što je kraće od vremena do prvog pokušaja podizanja i uspešnog ustajanja za blizance - odnosno 7,9 ± 1,3 min. i 28,3 ± 2 min. Jedinci su proveli skoro dva minuta više na nogama (P> 0,05) od blizanaca (22,6 ± 2,9 min. prema ± 20,9±2,3 min.). Muška i ženska jarad su svoj prvi pokušaj da se podignu imali skoro u isto vreme (ženke 6.8 ± 1.2 min i mužjaci 6.6 ± 1.1 min.) po rođenju. Vreme do prvog uspešnog pokušaja da ustanu je je bilo pod uticajem pola novo rođene jaradi - ženke su ustajale ranije nego mužjaci u proseku sa 6 minuta (29,8 ± 2,5 min za mužjake i 23,8 ± 1,6 minuta za ženke). Ženska jarad su stajali duže vreme na nogama u okviru prvog sata nakon rođenja (22.7 ± 2.6 min), ali je razlika u poređenju sa muškom jaradi (20.1 ± 2.4 min) nije dokazana. Jedinci su svoj prvi pokušaj da sisaju imali 25,7 ± 2.8 min i uspešno su sisali u proseku 33,0 ± 4,2 minuta nakon rođenja, što je ranije u poređenju sa blizancima, odnosno 31,8 ± 2,5 i

40,3 ± 3,2 min. Primećeno je da su ženska jarad imala prvi pokušaj da sisaju ranije (28.6 ± 2.9 min.), ali su počeli kasnije da sisaju (39,3 ± 3,4 min.) i proveli su manje vremena u sisanju tokom prvog sata nakon rođenja (6,3 ± 1,8 min.) u poređenju sa mužjacima (31,3 ± 2,5 min.; 34.4 ± 3,5 min. i 7.6 ± 2.5 min.).

Cilj ove studije bio je da prati ekspresiju ponašanja jaradi tokom ranog neonatalnog perioda.

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IMPORTANT MEAT QUALITY TRAITS OF MALE SIMMENTAL AND CHAROLAIS CROSSES

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Original scientific paper

Abstract: The paper presents the results of the most important meat quality traits of domestic Simmental breed (A) and its crosses with Charolais breed (B). The sample included a total of 30 animals, 15 in each group. Young bulls were slaughtered at the same age with an average weight of around 660 kg in both groups. Warm carcass sides, with and without kidney fat, were weighed immediately after slaughtering. After cooling, the left carcass sides were cut into main parts according to the Rulebook ("*Off. Gazette of SFRY", No. 34/74, 26/75, 13/78 - Regulations, 1/81 - Regulations and 2/85 - Regulations*) and the three rib cut (9-10-11 rib) separated from the back part. The results of the dissection show that young bulls of group (B) achieved a statistically significantly ($p < 0.05$) higher share of *M. longissimus dorsi*, while the share of body fat was significantly higher in young cattle of group (A) by 4.43. The chemical composition of *M. longissimus dorsi* differed significantly ($p < 0.05$) only in the share of protein, which was higher in young cattle of group (A). Young bulls of group (A) had statistically ($p < 0.05$) higher content of total pigments.

Key words: meat quality, *M. longissimus dorsi*, technical characteristics

Introduction

Beef production has been in slight increase, since 2001, but still does not meet the needs both in terms of number of animals, and in terms of productivity *Aleksić et al., 2007)*. Beef meat, in regard to the consumption of different kinds of meat, is in third place (21%), after pork (40%) and poultry (31%), which corresponds to the structure of consumption in countries with low overall meat consumption (below 50 kg per capita) in comparison to other countries with a consumption of over 100 kg (US, Australia, Canada, etc.), where meat of bovine animals has the largest share in the consumption structure (*Petričević et al., 2011*). Share of tissues, primarily meat, in retail cuts is defined by their quality, value and

market demand. Information on the content of tissues in main carcass parts that are available in the literature are mainly related to a particular anatomical region of the carcass, with large variations in terms of cutting of carcass sides and heterogeneity of the material used in the study (breed, gender, age, level of fattening, etc.) *(Petričević et al., 2011)*.

Satisfying the consumer's requirement for a consistent satisfactory product is the major target of beef producers and retailers. Meat quality is an important criterion that influences the decision of a consumer to purchase beef *(Sami et al., 2004)*. Besides slaughtering conditions and technological considerations, meat characteristics depend directly on the muscle biology of live animals, which is regulated by genetic, nutritional and biological factors. Among the latter, the genetic factors are of prime importance because genetic improvement is permanent and cumulative when inherited by the next generations. The genetic factors can be demonstrated by comparing different breeds *(Hocquette et al., 2005)*.

Serbia should in the future provide, quickly and efficiently, adequate quantities of top quality meat. Cattle production in the EU is tailored to consumer tastes. Meat must have a bright red color, equally distributed fatty tissues with desirable sensory characteristics such as tenderness, succulence, flavor and aroma. Results of the quality of beef crosses F1 generation (domestic spotted breed with French beef cattle breeds Charolais and Limousin) indicate that industrial crossing can improve fattening, slaughter and sensory characteristics of meat *(Aleksić et al., 2013)*. Cattle of domestic Simmental breed as the basis for crossbreeding with specialized beef cattle breeds is the fastest and most economical way to improve the fattening and slaughter traits *(Miščević et al., 2003; Bogdanović et al., 2005)*. French fattening/meat breeds - Charolais and Limousine, are characterized by favorable fattening and slaughter traits, as well as good quality of meat with a low fat content in carcasses, which is why these breeds can be fattened to higher final weights *(Ostojić-Andrić et al., 2007)*. In this regard, this paper aims to review the effect of genetic factors on different quality traits of beef (especially sensory quality and healthiness).

Materials and Methods

The experiment was conducted at the experimental farm of the Institute for Animal Husbandry (Belgrade, Serbia). Two groups of male cattle were formed: group A (n = 15) domestic Simmental breed and group B (n = 15) F1 generation crosses of Domestic Simmental breed with Charolais. Both groups of young bulls were fed *ad libitum* combined meal which consisted of a mixture of corn silage, according to the nutrition standard/tables, depending on the weight of the group. One day prior to slaughter young cattle were deprived of food, but had free access

to water. Slaughtering and primary processing of carcasses, cutting of carcasses and dissection of three rib cut were carried out in the experimental slaughterhouse and chemical composition and technological properties in the laboratory, of the Institute for Animal Husbandry. Three rib cut (9-10-11 rib) was separated from the left chilled carcass side at the cranial edge of the 9th and 11th rib and cut parallel to the spinal column. Using scales with an accuracy of 0.001 kg, muscle the masses of following tissues were measured: muscle tissue (especially *M. longissimus dorsi*), fat and connective tissue and bone. The chemical composition of the sample *M. longissimus dorsi* was determined (water content - the method of drying the sample at 103±2°C *(SRPS ISO 1442, 1998)*, the fat content - extraction method by Soxhlet *(SRPS ISO 1444, 1998)*, the amount of mineral matter (ash) - by burning the sample at 550±25 °C *(SRPS ISO 936, 1999)* and protein content - method according to Kjeldahl *(SRPS ISO 937, 1992)*. Technological properties of the sample *M. longissimus dorsi* and pH value of meat 45 minutes, 24 hours and 48 hours post mortem were measured using pH meter with combined puncture electrode Hanna HI 83141 (Hanna Instruments, USA), cooking loss was determined on the basis of the difference in weight of the pieces meat (size: 3 x 4 x 1.5 cm, and weight about 70 g) before and after cooking in distilled water (wherein the ratio of meat and water was 1:2) in a closed glass vessel (at 100°C for 10 min) and is expressed as a percentage relative to the weight of the sample before cooking *(Off. Gazette no. 2/85, 12/85 and 24/86)*, roasting loss was determined on the basis of the difference in weight of the pieces of meat before and after roasting. Cut/filet of *M. longissimus dorsi*, which was cut transversely to the direction of muscle fibers, weighing 150±1 g was wrapped in aluminum foil and roasted for 25 minutes at 250°C. After completing the roasting it was extracted from the aluminum foil and immediately measured.

The softness (tenderness) of meat was determined according to Volodkevich (1938) by cutting pieces of meat transverse to the direction of the muscle fibers. Determination of total pigments was performed by method of Horsney *(Bunning and Hamm, 1970)* and instrumental color measurement was done using Chroma Meter CR-400 (Minolta, Japan), which had been previously calibrated in relation to a standard white surface (illumination D65, viewing angle 20° and opening of the probe 8 mm) on fresh meat samples (24 hours post-mortem). Samples of meat were cut and left 30 minutes in air to stabilize colors. Color values are represented in the CIE L*a*b* system *(CIE, 1976)*, where L* is a measure indicating lightness of meat, and a* indicating the relative share of red and b* relative share of yellow. For each sample of meat three readings were carried out and their mean value was used for statistical data processing. Cross-sectional area of *M. longissimus dorsi* was determined on cross section of *M. longissimus dorsi* in the region of 11th rib by tracing on the tracing paper, and then measuring

using the planimeter. Evaluation/scoring of marbling of meat was done on fresh samples of meat by visual assessment at the intersection of *M. longissimus dorsi* and taste, aroma, succulence and tenderness of meat were scored after cooking and after roasting. For each evaluated parameter quantitative descriptive scale of 5 points was used; marbling: 1 - very poor marbling, 2 - poor marbling, 3 - neither good nor poor marbling, 4 - good marbling, 5 - very good marbling; taste and odor: 1 - very poor, 2 - poor, 3 - neither good nor poor, 4 - good, 5-very good; Softness: 1 - very firm, 2 - firm, 3 - neither firm nor soft, 4 - soft, 5 - very soft; succulence: 1 - very dry, 2 - dry, 3 - neither dry nor succulent, 4 - succulent, 5 - very succulent.

The obtained data were analyzed using analysis of variance in single factorial experiment (One-way ANOVA) by SPSS Statistics 20. The statistical significance of differences between mean values was determined by t-test.

Results and Discussion

Table 1 shows the shares of certain tissues in three rib cut. Share of *M. longissimus dorsi* differed significantly between groups ($p < 0.05$). Higher share of *M. longissimus dorsi* was determined in young cattle of group (B) by 7.07% compared to the group (A). The share of muscle tissue (*M. longissimus dorsi* + other meat) in group (B) was 72.55%, and in group (A) 67.40%. A statistically significant difference was found in the share of fat tissue ($P<0.05$), which was higher in young cattle of group (A). Similar results are reported by *Aleksić et al. (2005)* who stated in their study that the share of fatty tissue of three rib cut was 8.34% and 18.77% share of bones of young cattle of Domestic Simmental breed.

Taking into consideration the higher share of the muscle tissue, lower share of the fatty tissue and bones the advantage in our study was on the side of young cattle of group (B).

Table 1. Shares of different tissues in three rib cut

Item	A	B	t-test
Three rib cut (%)			
M. longissimus dorsi	36.04±4.76	43.11±6.47	*
Other muscle tissue	31.36±1.74	29.44±5.89	ns
Fat tisue	14.06±2.79	9.63±2.94	*
Connective tissue	0.90±0.37	1.15±0.31	ns
Bones	17.64±4.03	16.67±3.75	ns

ns – not significant
* significant at the level of ($p<0.05$)

The chemical composition of *M. longissimus dorsi* is shown in Table 2. The water content and ash content were higher in young cattle of group (B), but the

difference was not statistically significant. A statistically significant difference was found in protein content, which was higher in young cattle of group (A). The study by *Aleksić et al. (2013)* shows similar results and states that the content of proteins in the *M. longissimus dorsi* of crossbred beef cattle of Domestic Simmental and the Charolais breed was 22.10%. *Štoković et al. (2009)* in their work give following values: share of water 75.13%, fat 3.14%, ash 10.01% and protein 20.63% for young cattle of Domestic Simmental breed.

Table 2. Chemical composition of *M. longissimus dorsi*

Item	A	B	t-test
Water, (%)	74.10±1.44	75.66±1.06	ns
Lipid, (%)	1.77±0.66	1.34±0.55	ns
Ash, (%)	1.07±0.037	1.10±0.02	ns
Protein, (%)	23.05±1.07	21.87±0.58	*

ns – not significant
* significant at the level of ($p<0.05$)

Table 3 shows the technological characteristics of *M. longissimus dorsi*. A statistically significant difference was found only in the content of total pigments that was higher in young cattle of group (A) ($p <0.05$). In respect of other technological characteristics of *M. longissimus dorsi*, no significant differences were established. In the research of *Petričević et al. (2013)* the value of meat cooking loss of 37.10% is reported, as determined for young cattle of Domestic Simmental breed.

The softness of the meat was better, i.e. the meat was softer in young cattle of group (B), but the difference was not statistically significant. Similar results were obtained by *Bureš and Bartoň (2012). Aleksić et al. (2013)* report that the value of softness (tenderness) of meat indicated that the meat of young cattle of Domestic Simmental breed crossbred with Charolais was softer and that the muscle fibers were of smaller diameter compared to young cattle of Domestic Simmental breed. Color of meat obtained from crosses of Domestic Spotted breed with Charolais and Limousine was more prominent compared to meat of Domestic Spotted cattle, and these differences ($p<0.05$) were statistically significant, according to *Aleksić et al. (2013).* The research results of *Aleksić et al. (2013)* show that domestic Simmental beef cattle have a greater surface section *M. longissimus dorsi* in relation to crosses of Domestic Simmental with Charolais. These data coincide with the results of these investigations.

Table 3. Technological properties of *M. longissimus dorsi*

Item	A	B	t-test
pH			
45 min	6.63±0.16	6.72±0.06	ns
24h	5.60±0.08	5.67±0.10	ns
48h	5.71±0.36	5.66±0.04	ns
Cooking loss, %	42.59±1.04	42.28±1.55	ns
Roasting loss, %	41.00±1.99	42.99±0.79	ns
Tenderness, kg	10.44±2.69	9.47±2.86	ns
Total pigments, mg/kg	184.88±29.77	136.50±31.20	*
Meat color	36.47±0.65	36.01±0.13	ns
Cross section	103.83±9.58	94.60±45.32	ns
Marbling	2.09±1.04	1.60±0.55	ns

ns – not significant
* significant at the level of ($p<0.05$)

Softness (texture) and succulence of cooked or roasted meat, and to a certain extent, aroma and taste, are important parameters of meat quality. Table 4 shows the sensory characteristics of cooked and roasted meat. Statistically significant differences were not recorded between studied parameters. Sensory evaluation (odor, taste, tenderness, succulence) of cooked and roasted meat was practically the same in both groups of cattle (average cumulative score), group (A): cooked meat – 4.32 and roasted meat - 3.82; Group (B): cooked meat – 4.04 and roasted meat - 3.91. Differences in sensory characteristics may be attributed to the different content of intramuscular fat *(Christensen et al., 2011).* Research by *Chambaz et al. (2003)* confirms that there are no differences in the sensory characteristics of *M. longissimus dorsi* in young cattle of Domestic Simmental and Charolais breeds.

Tabela 4. Sensory properties of *M. longissimus dorsi*

Item	A	B	t-test
Cooked meat			
Odor	4.68±0.34	4.67±0.58	ns
Taste	4.55±0.47	4.33±0.76	ns
Tenderness/softness	4.10±0.54	3.67±0.76	ns
Succulence	3.95±0.42	3.50±0.50	ns
Roasted meat			
Odor	4.25±0.35	4.33±0.29	ns
Taste	4.27±0.34	4.17±0.29	ns
Tenderness/softness	3.50±1.16	3.67±0.58	ns
Succulence	3.27±1.03	3.50±0.50	ns

ns – not significant

Conclusion

Based on the results of the investigation of the possibilities of improving the quality of meat obtained by crossing domestic Simmental beef cattle with the French breeds such as Charolais, the following can be concluded:

- Young bulls of group (B) achieved a statistically significant ($p<0.05$) higher share of *M. longissimus dorsi* and significantly ($p<0.05$) lower share of fat tissue;
- Young bulls of group (B) had lighter color meat and statistically significant ($p<0.05$) lower content of total pigments;
- Major technological and sensory properties of meat were the same;
- Young bulls of group (A) had statistically significant ($p<0.05$) more proteins in the *M. longissimus dorsi*.

Based on this we can conclude that the crossing of Domestic Simmental breed and Charolais resulted in a significant improvement in the yield of meat without a negative impact on the quality.

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Važnije osobina kvaliteta mesa muške junadi simentalske rase i meleza sa šaroleom

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Rezime

U radu su prikazani rezultati ispitivanja važnijih karakteristika kvaliteta mesa domaće simentalske rase (A) i njenih melaza sa šarole rasom (B). Uzorkom je obuhvaćeno ukupno 30 grla, po 15 u svakoj grupi. Junad su zaklana u istom uzrastu sa prosečnom težinom oko 660 kg u obe grupe. Nakon klanja izvršeno je pojedinačno merenje toplih polutki sa i bez bubrežnog loja. Posle hlađenja leva polutka je rasecana u osnovne delove prema pravilniku ("Sl. list SFRJ", br. 34/74, 26/75, 13/78 – dr. pravilnik, 1/81 – dr. pravilnik i 2/85 – dr. Pravilnik) i iz leđnog dela izdvojen je trorebarni isečak (9-10-11 rebro). Dobijeni rezultati disekcije

pokazuju da su junad grupe (B) ostvarila statisticki značajno ($p<0.05$) veći udeo *M. longissimus dorsi*, dok udeo masnog tkiva je bio statistički značajno veći kod junadi grupe (A) i to za 4.43. Hemijski sastav *M. longissimus dorsi* se statistički značajno razlikovao ($p<0.05$) samo u udelu belančevina, koji je bio veći kod junadi grupe (A). Junad grupe (A) su imala statistički ($p<0.05$) veći sadržaj ukupnih pigmenata.

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A COMPARATIVE STUDY ON THE FATTY ACID PROFILE OF THREE SERBIAN TRADITIONAL DRY-FERMENTED SAUSAGES

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Abstract: In this study fatty acid composition of three traditional dry-fermented sausages (*Lemeški kulen, Sremski kulen* and *Petrovská klobása*) from Vojvodina (northern Serbia) was investigated. The fatty acid composition of *Lemeški kulen* was found to be approximately 35.67% saturated fatty acids (SFA), 47.0% monounsaturated fatty acids (MUFAs) and 17.33% polyunsaturated fatty acids (PUFAs), whereas the fatty acid profile of *Sremski kulen* consisted of 38.63% SFAs, 46.58% MUFAs and 14.77% PUFAs. Statistically significant difference ($p<0.05$) was noticed in the content of SFAs (35.36%) in *Petrovská klobása* compared to SFAs content (38.63%) in *Sremski kulen*, while MUFAs and PUFAs were found to be around 47.86% and 16.78%, respectively. Based on the obtained results it could be concluded that fatty acid profiles of investigated traditional dry-fermented sausages are basically comparable, with the small mismatch in SFAs contents between *Sremski kulen* and *Petrovská klobása.*

Key words: fatty acid composition, dry-fermented sausage, *Lemeški kulen, Sremski kulen, Petrovská klobása*

Introduction

Traditional food is an important part of the cultural identity of European peoples and regions, and its production and sales are of substantial economic income, due to the growing consumer interest in typical products whose quality is closely linked to geographical origin and traditions *(Škaljac, 2014)*. In many European countries, including Serbia, a large number of fermented sausages are produced, whose peculiar characteristics originate from different raw materials, recipes and production processes that are result of habit and custom of the people *(Ikonić et al., 2010)*.

Kulen represents dry-fermented sausage which is traditionally produced during the winter period in areas known for manufacturing pork and red hot paprika, such as northern Serbia (Srem and Bačka), eastern Croatia (Slavonia and Baranja) and southern Hungary *(Vuković, 2006; Vuković et al., 2011)*. Depending on location of production, each sausage has an appropriate name, for example, *Sremski kulen*, *Lemeški kulen*, *Baranjski kulen*, *Petrovská klobása* etc. *(Vuković et al., 2012)*. *Lemeški kulen* is dry-fermented sausage made from minced high-quality meat of mature pigs, with the addition of red paprika powder. The way of grinding the meat and the amount of red paprika added makes this sausage different from other, similar products of Pannonian Plain. *Sremski kulen* is a fermented sausage from Srem dictrict in Vojvodina (northern Serbia) and it is characterized by a high level of proteins and low level of fat and moisture. It is traditionally made from the meat of local, late maturing pigs of the Mangulica breed. Since this breed had almost disappeared, nowdays *Sremski kulen* is produced from the meat of modern pig breeds *(Živković et al., 2012)*. *Petrovská klobása* is made in area nearby town of Bački Petrovac in northern Serbia, as a part of Slovak's heritage. This traditional sausage is characterized by specific savory taste, aromatic and spicy-hot flavor, dark red color and hard consistency *(Ikonić et al., 2010)*.

In recent years, the great attention is directed towards fatty acid composition considering that lower saturated fatty acid (SFA) content and higher proportion of unsaturated fatty acid (USFA) are advantageous from a human nutrition point of view *(Holló et al., 2003)*.

The aim of the present study was to investigate fatty acid profile and to detect potential differences between traditional dry-fermented sausages manufactured in province of Vojvodina (northern Serbia). *Lemeški kulen*, *Sremski kulen* and *Petrovská klobása* were selected as the most appreciated and widespread traditional sausages in this region.

Materials and Methods

Three Serbian traditional dry-fermented sausages were considered in this study: (a) *Lemeški kulen*, manufactured in the northwestern part of Vojvodina province (Svetozar Miletić village, Municipality of Sombor), (b) *Sremski kulen*, manufactured in the Srem region, area nearby towns of Sremska Mitrovica and Šid, and (c) *Petrovská klobása*, manufactured in the western part of Vojvodina province (Municipality of Bački Petrovac). The procedures of manufacture are shown in Figure 1. The sausages are generally supplied on the market after 4-6 months of drying/ripening process. The weight of the *Petrovská klobása* sausages was 500-600 g and the diameter ranged from 40 to 50 mm, while the weight of *Lemeški kulen* and *Sremski kulen* was 700-800 g and the diameter varied from 70 to 90 mm. The sample sausages were supplied in triplicate (different batches) by local traditional producers and were stored in vacuum package at 4^0C before analyses. All the analyses were carried out in duplicate for each batch.

Lipids were extracted from the samples of 1 gram by using cold extraction process, which involves mixing/homogenizing with chloroform: methanol mixture (2:1) according to the method by *Folch et al. (1957)***.**

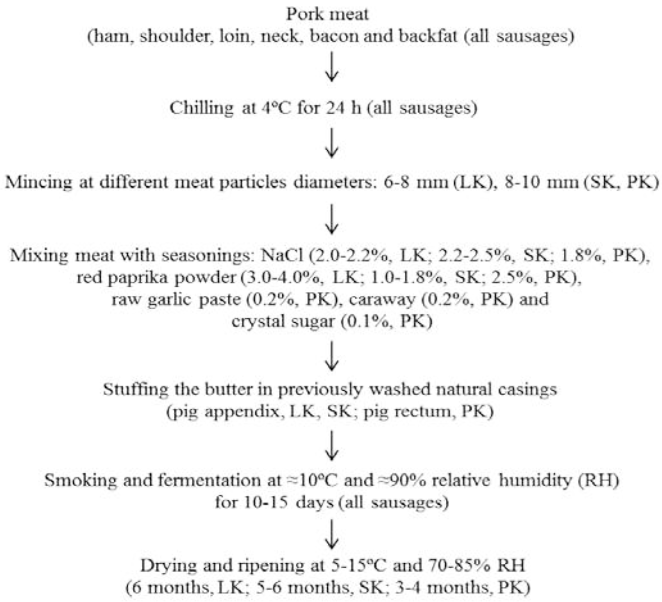

Figure 1. Procedures for the manufacture of the three Serbian dry-fermented sausages. LK - *Lemeški kulen*; SK – *Sremski kulen*; PK - *Petrovská klobása*.

Fatty acid methyl esters were prepared from the extracted lipids using method based on 14% boron trifluoride/methanol solution, as recommended method for this type of substrates *(Verešbaranji, 1996)*. Nitrogen gas was used for drying and removing solvents from fatty acid methyl esters.

Obtained samples were analyzed by a GC Agilent 7890A system with FID, autoinjection module for liquid, equipped with fused silica capillary column (SP-2560, 100 m x 0.25mm, I.D., 0.20μm). Helium was used as a carrier gas (purity > 99.9997 vol %, flow rate = 1.26 ml/min).

The fatty acids peaks were identified by comparison of retention times with retention times of standards from Supelco 37 component fatty acid methyl esters mix and with data from internal data library, based on previous experiments. Results were expressed as mass of fatty acid or fatty acids group (g) in 100 g of fatty acids.

Statistical analyses were conducted using the statistical package program *Statistica 12 (2013)* to determine if variables differed between samples. Significant effects were explored using analysis of variance (ANOVA) and Fisher's LSD post-

hoc test to ascertain differences among sample means. A significance level of $p<0.05$ was used.

Results and Discussion

Regarding the consumer concern with the saturated/unsaturated fatty acid ratio in the diet, the fatty acid profile of meat products has lately received particular attention. The fatty acid compositions found in the investigated samples of dry-fermented sausages are shown in Table 1.

Table 1. Fatty acid composition of traditional dry-fermented Serbian sausages

Fatty acid	Type of sausage		
	Lemeški kulen	*Sremski kulen*	*Petrovská klobása*
C4:0	0.35±0.27^{ab}	0.51±0.02^{b}	0.06±0.03^{a}
C6:0	ND	ND	0.01±0.02
C8:0	ND	ND	0.01±0.01
C10:0	0.04±0.04	0.07±0.01	0.06±0.01
C12:0	0.06±0.05	0.09±0.02	0.08±0.01
C14:0	1.29±0.04	1.43±0.11	1.33±0.08
C14:1	0.01±0.01	0.02±0.03	0.01±0.01
C15:0	0.07±0.05^{ab}	0.13±0.03^{b}	0.05±0.01^{a}
C16:0	22.37±0.99	23.85±1.33	22.04±0.96
C16:1	2.38±0.42	2.57±0.64	2.26±0.29
C18:0	11.22±0.53	12.29±0.95	11.41±1.15
C18:1n9c	43.87±6.41	43.21±1.91	44.80±0.50
C18:2n6c	14.60±6.14	12.28±0.70	14.53±1.37
C20:0	ND	ND	0.06±0.02
C18:3n6	0.08±0.07	0.08±0.07	0.10±0.02
C20:1n9	0.56±0.31	0.51±0.10	0.64±0.06
C18:3n3	1.17±0.23	0.86±0.18	1.13±0.09
C20:2	0.58±0.20	0.47±0.10	0.66±0.12
C22:0	0.27±0.05	0.38±0.16	0.24±0.02
C22:1n9	ND	0.01±0.01^{a}	0.05±0.03^{b}
C20:3n3	0.89±0.31	1.07±0.56	0.34±0.13
C20:4n6	ND	ND	0.01±0.01
C22:2n6	0.01±0.02	0.01±0.02	ND
C24:0	ND	0.03±0.06	0.03±0.03
C24:1	0.18±0.08	0.27±0.13	0.11±0.02
C22:6n3	ND	ND	0.01±0.02
ΣSFAs	35.67±0.64^{ab}	38.63±1.32^{b}	35.36±2.12^{a}
ΣMUFAs	47.00±6.44	46.58±1.57	47.86±0.68
ΣPUFAs	17.33±6.69	14.77±0.52	16.78±1.46

Values are expressed as percentages of total fatty acids; values are given as mean ± SD (n=6);
$^{a-b}$Means within a row with no common superscript differ significantly at $p<0.05$; ND- not detected.

The fatty acid composition of *Lemeški kulen* consisted of approximately 35.67% saturated (SFA), 47.0% monounsaturated (MUFAs) and 17.33% polyunsaturated acids (PUFAs). Similarly, the fatty acid profile of *Sremski kulen* was found to be 38.63% saturated (SFA), 46.58% monounsaturated (MUFAs) and 14.77% polyunsaturated acids (PUFAs). Analysis of *Petrovská klobása* showed significantly different ($p<0.05$) content of saturated fatty acid (35.36%) to SFA content (38.63%) in *Sremski kulen*. Furthermore, monounsaturated (MUFAs) and polyunsaturated acids (PUFAs) in *Petrovská klobása* were found to be around 47.86% and 16.78%, respectively.

Palmitic acid (C16:0) was the most abundant SFA, while oleic acid (C18:1n9-cis) was the most common of the monounsaturated fatty acids (MUFAs) in all investigated samples. The consumption of MUFAs, especially oleic acid, has been shown to decrease plasma triacylglycerol and cholesterol concentrations, without any adverse effect regarding the plasma HDL-cholesterol levels in healthy normolipidaemic subject *(Baro et al., 2013).* Regarding the polyunsaturated fatty acids (PUFAs) it was noticed that linoleic acid (C18:2n6-cis) was the most frequent for all types of traditional sausages. Linoleic acid is one of essential fatty acids since human body cannot synthesize it and therefore is of great interest consumption of food with high levels of this acid *(Schmitz and Ecker, 2008).* Linoleic acid plays an important role as precursor in the biosynthesis of arachidonic acid (AA) and thus some prostaglandins, leukotrienes (LTA, LTB, LTC) etc., which are important regulators of cellular functions with inflammatory, atherogenic and prothrombotic effects *(Schmitz and Ecker, 2008).* As it can be seen from the Table, significant difference ($p<0.05$) was noticed in butyric acid (C4:0) and pentadecanoic acid (C15:0) content between *Sremski kulen* and *Petrovská klobása* samples. On the other side, erucic acid (C22:1n9) content in *Petrovská klobása* was significantly higher ($p<0.05$) compared to the content in *Sremski kulen*. This differences led to significantly higher ($p<0.05$) SFA content in *Sremski kulen* compared to *Petrovská klobása.*

Conclusion

From reported findings, it can be concluded that the fatty acid profiles of investigated traditional dry-fermented sausages were quite similar, with a small but statistically significant difference in content of saturated fatty acid between *Sremski kulen* and *Petrovská klobása.* From a human health point of view, these traditional meat products have quite desirable content of unsaturated fatty acids compared to saturated fatty acids, since it is known that nutritionists recommend a reduction in SFA and trans fatty acids, which are associated with an increased risk of cardiovascular disease and some cancers.

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Komparativna studija masnokiselinskog sastava tri tradicionalne suve fermentisane kobasice iz Srbije

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Rezime

U ovom radu je ispitan masnokiselinski sastav tri tradicionalne suve fermentisane kobasice (*Lemeški kulen, Sremski kulen, Petrovačka kobasica*) iz Vojvodine (severna Srbija). Ispitivanja su pokazala da *Lemeški kulen* sadrži oko 35,67% zasićenih, 47,0% mononezasićenih i 17,33% polinezasićenih masnih kiselina, dok sastav masnih kiselina u *Sremskom kulenu* čini 38,63% zasićenih, 46,58% mononezasićenih i 14,78% polinezasićenih kiselina. Statistički značajna razlika ($p<0.05$) primećena je u sadržaju zasićenih masnih kiselina (35,36%) u *Petrovačkoj kobasici* u odnosu na sadržaj zasićenih masnih kiselina (38,63%) u *Sremskom kulenu*, dok je sadržaj mononezasićenih i polinezasićenih masnih kiselina iznosio 47,86% i 16.78%, respektivno. Na osnovu dobijenih rezultata može se zaključiti da su masnokiselinski sastavi ispitivanih tradicionalnihfermentisanih kobasica prilično slični, sa malom razlikom u sadržaju zasićenih masnih kiselina između *Sremskog kulena i Petrovačke kobasice.*

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SENSORY EVALUATION AND QUALITY OF TRADITIONAL SMOKED MEAT PRODUCTS FROM SERBIA AND MONTENEGRO – SIGNIFICANCE FOR CONSUMERS

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Review paper

Abstract: This paper reviews researches published in recent years (2005-2015) concerning sensory properties and quality of smoked meat and meat products from Serbia and Montenegro. The newest results are presented as well. These researches are results of long and a successful collaboration between Federal Centre for Meat Research, Kulmbach, Germany (now Max Rubner-Institute) and Institute of Meat Hygiene and Technology, Belgrade, Serbia. Sensory evaluation of the products was done according to the DLG-5-point-testing-scheme by experts in Kulmbach as well as participating in the official international DLG quality tests in Germany. Traditional smoked meat products from Serbia and Montenegro showed satisfactory sensory evaluations. 57% of the analysed samples (smoked beef, pork and sheep ham and poultry salami, n=155) were evaluated with DLG award in Gold, 31% in Silver, while 12% of smoked ham were evaluated in DLG award in Bronze. At consumers' point of view, traditional smoked meat and meat products from Serbia and Montenegro, would be enrichment for the (West) European market. High salt content and too strong smoke of the meat products are the main deficiencies.

Key words: sensory evaluation, traditional, smoked meat products, consumer

Introduction

Meat and meat products are important sources of proteins, trace elements and vitamins and its consumption has been important in human species evolution, especially the brain and intellectual development *(Pereira and Vicente, 2013)*. For consumers, sensory properties of meat and meat products are very important *(Font-*

I-Furnols and Guerrero, 2014). With the purchase of food German consumers look for security and orient themselves to quality awards. The average consumption of meat products in Germany in 2013 were shown in Figure 1 *(DFV, www.fleischerhandwerk.de, 2015).* According to one industrial slaughterhouses in Serbia *(Glamočlija et al., 2015)*, Serbian sausage was the most represented sausage (33.05%), followed by cooked (24.23%) and homemade sausage (17.12%). The most represented smoked products was dry bones (33.22), followed by dry meat (28.25%) and bacon (24.52%). Consequently, the percentage of production of the product is proportional to their consumption by Serbian population.

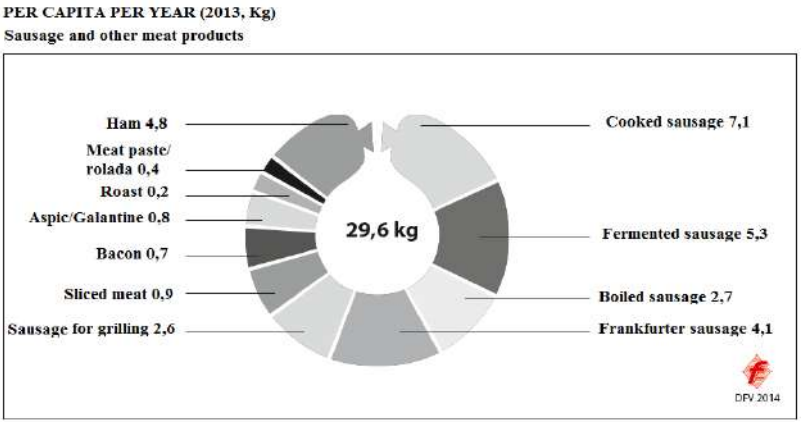

Figure 1. The average consumption of sausage and other meat products in Germany in 2013

This paper reviews researches published in recent years (2005-2015) and shows the newest results, concerning sensory properties and quality of smoked meat products from Serbia and Montenegro. These researches are results of long and a successful collaboration between Federal Centre for Meat Research, Kulmbach, Germany (now Max Rubner-Institute) and Institute of Meat Hygiene and Technology, Belgrade, Serbia. Additionally, the aim of this study was to determine whether the traditionally smoked meat products from Serbia and Montenegro satisfy the German quality criteria, and accordingly, whether such products could be exported to the German market.

Physico-chemical properties of smoked meat

Quality of traditional smoked meat products were tested by performing different physico-chemical parameters (pH-value; a_w-value; peroxide value; acid value; content of water, protein, fat, ash, sodium chloride, nitrite, nitrate), *(Troeger et al., 2009).* Fatty acids composition and content of polycyclic aromatic hydrocarbons of some smoked meat products from Serbia (Zlatiborac, Mackat) were performed by applying GC/ECD and HRGC/MS/MS, respectively *(Djinovic,*

2008). Physico-chemical properties of different smoked meat and smoked meat products from Serbia (Zlatibor) were analysed in few common studies *(Troeger et al., 2009; Djinovic, 2008).* Table 1 shows properties of beef ham. The pH values were in the standard range for this type of product. The a_w values were between 0.886 and 0.933 and they provide product safety e.g. prevent growth pathogenic bacteria *(Leistner et al., 1981)*. The salt content of ham valued between 3 and 6.1%. According to the literature data the average salt content for these products is 4.5% *(Vukovic, 2012)*. Contents of nitrate (495 and 680 mg/kg) in 2 samples were higher than MDK value (250 mg/kg), while the nitrite content was in the standard range. Mean content of benzo(a)pyrene (1.7 µg/kg) was below MDK value (2 µg/kg).

Table 1. Physical properties, curing salt and Benzo(a)pyrene content of raw cured beef (beef ham, n=17) (Troeger et al., 2009)

Statistical parameters*	pH-value	a_w-value	NaCl [%]	Nitrite [mg/kg]	Nitrate [mg/kg]	Benzo[a]pyrene [µg/kg]
Mean	5.54	0.903	4.9	13	227.5	1.7
SD*	0.17	0.021	1.13	10.8	234.9	0.16
Min	5.38	0.886	3	1	37	1.5
max	5.77	0.933	6.1	33	680	1.8

*Statistical parameters expressed as standard deviation (SD), mean, maximum (max) and minimum (min) value.

According to *Djinovic (2008)* the protein content of beef ham was between 28.9% and 38.1%, while the fat content was between 7.2% and 10.1%. The results for raw hams from Montenegro (Njegusi, Montenegro) showed that NaCl content was between 7.87% and 9.05% while this content in fermented sausage was lower (from 3.52% to 5.75%), *(Troeger et al., 2006)*. The content of nitrate in some smoked samples from Montenegro was higher than MDK value and was between 153.9 mg/kg and 562.8 mg/kg. This can be explained by the high nitrate content (2723 ppm) in used sea salt. The other physical and chemical parameters were in the standard range *(Troeger et al., 2006)*.

Sensory evaluation

German Agricultural Society (DLG) that promotes product quality of foods, every year in Germany, organizes the DLG Quality Tests. The "DLG Award Winner" label is only issued to food that have been found worthy of it in sensory testing and that satisfy the DLG's stringent quality criteria. Sensory evaluation of the products from Serbia and Montenegro was done according to the DLG-5-point-testing-scheme (DIN ISO/IEC 17065, 2013; DIN ISO/IEC 17024, 2012). DLG Award could be achieved as: DLG award in Gold (excellent, quality figures 5), DLG award in Silver (very good, quality figures 4.6-4.99), DLG award in Bronze (good, quality figures 4.1-4.59) and without DLG award (quality figures <4.00), *(DLG Certification Unit, 2014)*.

The products delivered from Serbia (n=146) and Montenegro (n=9) (smoked beef, pork, sheep products, and poultry salami) have reached above-average DLG evaluations (in gold 57 % , silver 31 % and bronze 12 %), (Figure 2.). The sensory evaluations revealed deviations of the fat taste in some products, which were correlated with a higher level of polyunsaturated fatty acids. That probably indicates on unsuitable raw material. On the other hand, sensory evaluation of hams from Čajetina, named "Dalmatinski prsut", resulted in high resp. highest ratings *(Troeger et al., 2007)*. The main deficiencies of sensory properties for row cured meat referred to the external appearance i.e. external preparation (poorly dressed-remarks samples, 9-10%), consistency (too soft samples, 7-8%), odor and taste (salty samples, 14-15%), *(Stiebing et al., 2014)*. Type of smoking can cause the mentioned deficiencies of smoked meat products.

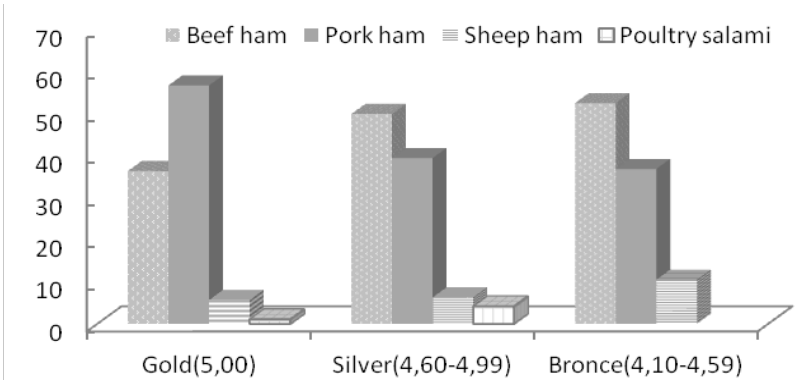

Figure 2. Sensory evaluation of smoked ham occurrence [%] of quality numbers

The newest results with Serbian consumers, concerning sensory properties of different traditional products was carried out during 81st International Agricultural Fair in Novi Sad, Serbia (Table 2).

Table 2. Results of consumers' sensory evaluation of salami, sausage and chicken salami during International Agricultural Fair in Novi Sad, Serbia, 2014 (n=85, rating in %)

	Taste % (n)			Salt content % (n)			Smoke (n)		
#	I*	II*	III*	I	II	III	I	II	III
3	55.3^{A,B} (47)**	63.5^{A,B} (54)	69.4^{A,B} (59)	83.5^{A,B} (71)	84.7^{A,B} (72)	88.2^{A,B} (75)	76.5^{A,B} (65)	82.4^{A,B} (70)	74.1^{A,B} (63)
2	31.8^{A,C} (27)	31.7^{A,C} (27)	29.4^{A,C} (25)	15.3^{A,C} (13)	5.9^{A} (5)	5.9^{A} (5)	16.5^{A} (14)	15.3^{A,a} (13)	20. 0^{A,a} (17)
1	12.9^{B,C} (11)	3.5^{B,C} (3)	1.2^{B,C} (1)	2.4^{B,C} (2)	9.4^{B} (8)	5.9^{B} (5)	7.1^{B} (6)	3.5^{B,a} (3)	5.9^{B,a} (5)

*I - Salami (100% beef meat); II - Sausage (20% beef meat and 80% pork meat); III - Chicken Salami (72% broiler breast meat). ** - Absolute number of the samples is shown in parentheses. # - rating level: Taste: 3- satisfactory, 2- average, 1- non satisfactory; Salt content: 3- balanced, 2- not salty enough, 1- too salty; Smoke: 3- balanced, 2- too weak, 1- too strong. Same letters between columns: ^{A,B,C} – $p<0.01$; ^{a} – $p<0.05$; (Chi-square test, *www.graphpad.com*).

The traditional smoked meat products were evaluated by 55.3-88.2% of interviewed consumers as products with satisfactory taste, balanced salt content and smoke. Results of statistical analyses showed that there is significantly difference ($p<0.01$) between consumers' frequency answers (satisfactory, average, non-satisfactory). Although same samples were evaluated as samples with too strong smoke, some investigations *(Troeger et al., 2009)* indicate that traditional smoked meat and meat products with strong smoke contain higher antioxidant constituents (phenols) in comparison with industrial smoked meat products. Those antioxidant constituents protect fat in meat products from oxidative and hydrolytic degradation process and consequently preserve the taste.

Concluding remarks

The obtained results admit the conclusion that traditional smoked meat products from Serbia and Montenegro showed satisfactory sensory evaluations, but at consumers' point of view, they would be enrichment for the (West) European market. High salt content and too strong smoke of the meat products are the main deficiencies. Additionally, beef steak and its' related products are not represented in the German market, but they could be, considering the fact that they were evaluated with the highest DLG awards.

Acknowledgment

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Senzorna ocena i kvalitet tradicionalno dimljenih proizvoda od mesa iz Srbije i Crne Gore – značaj za potrošače

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Rezime

U ovom radu dat je pregled istraživanja (2005-2015) kvaliteta i senzornih osobina dimljenog mesa i dimljenih proizvoda od mesa iz Srbije i Crne Gore. Takođe su prikazani i najnoviji rezultati istraživanja. Dobijeni podaci u ovoj studiji

su rezultat dugogodišnje i veoma uspešne saradnje između Federalnog centra za nauku o mesu (sada Max-Rubner Institut) iz Kulmbaha (Nemačka) i Instituta za higijenu i tehnologiju mesa iz Beograda (Srbija). Senzorna ocena proizvoda urađena je primenom svetski priznatog testa nemačkog poljoprivrednog društva (DLG test), kao i učešćem na zvaničnim DLG ocenjivanjima u Nemačkoj. Tradicionalno dimljeni proizvodi od mesa iz Srbije i Crne Gore bili su ocenjeni zadovoljavajućim senzornim DLG ocenama. Od 155 analiziranih dimljenih proizvoda, 57% je ocenjeno zlatnom DLG medaljom, 31% srebrnom, a 12% proizvoda je ocenjeno bronzanom DLG medaljom. I pored toga, sa aspekta potrošača sa zapadno evropskog tržišta, neophodno je poboljšati kvalitet tradicionalnih proizvoda iz Srbije i Crne Gore. Visok sadržaj soli i prejak dim su glavni nedostaci. Sa druge strane, na nemačkom tržištu nisu zastupljeni proizvodi poput goveđeg ramsteka i njemu sličnih proizvoda, a mogli bi da budu, s obzirom da su ocenjeni najvišim DLG ocenama.

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POSTER SECTION III

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EVALUATION OF GENETIC CONNECTEDNESS OF LANDRACE HERDS IN CZECH REPUBLIC

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Original scientific paper

Abstract: Production and reproduction data of the Czech Landrace pig breed were analysed. Degree of genetic connectedness between herds (measured and expressed as the connectedness rating; CR) was calculated by solving single trait (STAM) and two trait (TTAM) animal model. The average CR for one herd was calculated as its average connectedness with all other herds expressed in percentage. The CR within regions of Czech Republic was also evaluated. The genetic connectedness existed in all 51 of analysed herds. The CR varied from 0.1% to 54.7% for STAM and from 0.1% to 56.0% for TTAM. The average CR was 9.50%and 9.90% when applying STAM and TTAM, respectively. The 17.6% of herds reached the CR lower than 3% and this value was not affected by used model. Approximately one half of analysed herds have CR between 10 and 20%. On the other side, only 2.0% and 3.9% of herds (for STAM and TTAM, respectively) had CR higher than 20%. The high variability of CR among Czech regions was observed. The lowest CR was measured for herds in West Bohemia region (4.4%). Contrary, the highest CR was fund out for herds in East Bohemia and North Moravia regions (11.9% and 12.0%). Low values observed in last mentioned regions are probably due to low proportion of herds and relative small amount of animals. The connectedness between herds of Czech Landrace breed is on good level. Rising of genetic connectedness e.g. by using of higher proportion of common boars is still needed.

Key words: pig, management unit, genetic connectedness, regions, Landrace

Introduction

The modern genetic evaluation methods, like BLUP, enable estimation and comparison of breeding values for individuals kept in different environments

(herds). This assumption is correct only if some degree of genetic relationship between herds exists *(Kennedy and Trus, 1993)*. The accuracy of genetic evaluation is influenced by this degree of connectedness *(Mathur et al., 2002)*. The higher genetic connectedness between herds improves the accuracy of comparisonof breeding values of animals indifferent environments. A several methods have been already developed to evaluate the genetic relationship, e.g. expressed as degree of connectedness, between herds *(Kennedy and Trus, 1993; Lewis et al., 1999; Mathur et al., 2002; Fouilloux et al., 2008)* byimplication of various types of statistics methods.

The aim of this study was to estimate the connectedness rating between herds of Czech landrace pig breed population by using two animal models.

Material and Methods

Pedigree information, along with production and reproduction field test data of the Czech Landrace pig (dam breed) of the Czech national pig breeding program were analyzed. All data were provided by the Pig Breeders Association of the Czech Republic. Individual data sets (type of data and appropriate pedigree information) were defined to fully follow the traits included in the genetic evaluation of dam breeds in the Czech Republic *(Krupa and Wolf, 2013; Wolf et al., 2000)*.The average daily gain in the field test as a production trait and number of weaned piglets as a reproduction trait recorded for animals born in years 1995-2014 have been included in the analyses. In the pedigree data ten generation backwardwere included and the period from 1979 to 2014 was covered. Degree of connectedness between herds was analysed through solving of single trait (STAM) and two trait (TTAM) animal models. In the STAM effect of herd, year, sex, test type (fixed effects), animal and herd of origin (random effects) has been considered. For the TTAM the following effect: breed of boar, parity number, mating type, regression and permanent dam effect were additionally included. The regression was assumed as nested with parity number and was designed as follows: it contained linear and quadratic regression on age at first farrowing for first parity; otherwise it contained linear and quadratic regression on farrowing interval. The degree of genetic connectedness measured by connectedness rating (CR) *(Mathur et al., 1998)* was defined as the correlation between the management units (MU: herds, contemporary groups, etc.). Then the correlation coefficient between MU can be used to measure the genetic connectedness between pairs of MU by equation:

$$CR_{ij} = \frac{cov(\widehat{MU}_{ij})}{\sqrt{var(\widehat{MU}_i)var(\widehat{MU}_j)}}$$

where *i* and *j* represent two different MU. In large data sets the appropriate direct inverse of the entire set of mixed model equation is very difficult. Therefore, *Mathur et al. (1998)* used the procedure where the vector of inverse elements of one MU is obtained at a time through iteration method. The base result is triangular matrix of CR for all analysed herds, with one on diagonal and CRs on off-diagonal. The average CR for one herd was calculated as its average value obtained with all other herd expressed in percentage. The intervals of average CR was defined for better explanations of variability in this trait. All analysed herds were divided into seven groups by their affiliation to geopolitical regions in the Czech Republic. The basic characteristics of evaluated data are shown in Table 1. The DMU package *(Madsen et al., 2010)* was used to calculate the CR in our study. Further information about evaluated pig breed e.g. quality of pedigree, average inbreeding and co-ancestry, proportion of inbred animals and generation interval can be found in study *Krupa et al. (2015).*

Table 1. Basic characteristics of evaluated breed

	Single trait	Two trait
Number of animals in pedigree	132 483	132 109
Number of animals with data	165 765	126 080
Total number of herds	51	51
Average number of animals in herd	2 474	2 472
Proportion (%) of herds / animals in region:		
Middle Bohemia (MB)	11.8 / 13.0	11.8 / 13.3
South Bohemia (SB)	17.6 / 24.3	17.6 / 24.4
West Bohemia (WB)	7.8 / 2.0	7.8 / 1.8
North Bohemia (NB)	2.0 / 2.1	2.0 / 1.7
East Bohemia (EB)	21.6 / 31.1	21.6 / 32.3
South Moravia (SM)	23.5 / 15.5	23.5 / 15.3
North Moravia (NM)	15.7 / 12.1	15.7 / 11.2

Results and Discussion

The basic statistics of calculated connectedness rating (CR) for herds of Czech Landrace breed is summarized in Table 2. The direct or indirect genetic connectedness existed in all 51 of analysed herds. The degree of CR of one herd with other analysed herd varied in high extension, from 0.1%to 54.7%for single trait model (STAM) and from 0.1% to 56.0%for two trait model (TTAM). Generally, the type of data, methodology used to measure the connectedness rating and breed structure of herds should be especially mentioned and considered when our results with literature resources are compared.

Table 2. Basic statistics for connectedness rating (CR)

Variable	Single trait	Two trait
CR - mean	9.50%	9.90%
- standard deviation	5.50%	5.70%
- median	10.04%	10.42%
Proportion of herd with CR:		
≤ 3.0%	17.6%	17.6%
3.1% - 6.0%	15.7%	9.8%
6.1% - 10.0%	13.1%	15.7%
10.1% - 20.0%	51.0%	52.9%
> 20.0%	2.0%	3.9%

The average connectedness rating of Czech Landrace herds was 9.50% and 9.90% when solving the STAM and TTAM, respectively. Median values for appropriate models were a slightly higher. When the extra trait into the mixed model equations was added, the only negligible increase in the average connectedness of herds was found. In comparison, a low average connectedness rating (0 to 3.59%) of the herds was found by *Sun et al. (2009)* when analysing the connectedness between herds of three pig breed in China. They also stated, that the relatively high proportion of herds included in national swine improvement program till 2005 were not connected (44%, 85% and 72% of herds for Large White, Landrace and Duroc, respectively). A similar finding has been documented by *Xiao et al. (2010)*, who analysed connectedness between herds in Beijing China region. Low proportion of artificial insemination along with the relative small number of records and different performance testing structure could be the main reasons for differences observed between mentioned studies and our results. The average connectedness rating of herds at least 3% is recommended by *Mathur et al. (2002)* to reach the accurate comparisons of estimated breeding values for two traits in pig population. We assumed that the same criterion for average CR of herds can be recommended in our conditions. The proportion of herds with average CR split into five intervals is shown in Table 2. The 17.6% of herds reached the CR lower than 3% and this value was not affected by used model. Only a slightly increase in the average CR in subsequent intervals was found when TTAM was applied. Approximately for a half of analysed herds the CR ranged between 10 and 20%. On the other side, only 2.0% and 3.9% of herds had CR higher than 20% when using the STAM and TTAM, respectively.

Table 3. Distribution of herds among regions (%) according to the average connectedness rating when using the two trait model

Connectedness rating		Regions[a]						
		MB	**SB**	**WB**	**NB**	**EB**	**SM**	**NM**
≤ 3%	**Mean**	1.6	1.8	1.9	-	-	1.2	-
	Max.	1.6	3.0	2.0	-	-	1.8	-
	Min.	1.6	0.5	1.9	-	-	0.4	-
3% – 6%	**Mean**	5.3	-	-	-	4.1	3.1	-
	Max.	5.3	-	-	-	4.3	3.1	-
	Min.	5.3	-	-	-	3.8	3.1	-
6% – 10%	**Mean**	-	7.8	-	-	6.3	7.8	7.9
	Max.	-	8.1	-	-	6.3	9.6	9.7
	Min.	-	7.5	-	-	6.3	6.1	6.1
10% – 20%	**Mean**	12.5	13.1	11.9	10.3	15.2	14.3	13.4
	Max.	15.2	15.5	11.9	10.3	19.8	18.0	17.4
	Min.	10.5	10.4	11.9	10.3	11.2	10.2	10.3
>20%	**Mean**	-	20.3	-	-	21.3	-	-
	Max.	-	20.3	-	-	21.3	-	-
	Min.	-	20.3	-	-	21.3	-	-
Overall distribution		9.5	10.2	4.4	10.3	11.9	8.4	12.0

[a]MB – Middle Bohemia, SB - South Bohemia, WB-West Bohemia, NB-North Bohemia, EB-East Bohemia, NM-North Moravia, SM- South Moravia

Results of CR of herds among the regions of Czech Republic by using of TTAM are summarized in Table 3. The average CR of evaluated herds (along with minimum and maximum values) is divided into intervals for each region. Generally, the high variability of CR among Czech regions was observed. The lowest CR reached the herds in West Bohemia region (4.4%), contrary; the highest CR was fund out for the herds in East Bohemia and North Moravia region (11.9% and 12.0%). LowCR observed for herds in West and North Bohemia regions are probably due to low proportion of these herds and thus the animals farmed in these regions on the overall population of Landrace breed in Czech Republic (see in Table 1). A difference in CR of herds among individual regions has been also recorded by *Sun et al. (2009).*

Conclusion

The degree of genetic connectedness between herds of Czech Landrace pig breeds are on the good level. All of the analysed herds were directly or indirectly connected. The minimum criterion of 3% for connectedness rating (CR), suggested for reasonable comparison of breeding values between different environments, achieved more than 82% of analysed herds. When multitrait model was implicated,

the slightly higher CR of herds was found. It is still needed to increase genetic connectedness by using common boars.

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Procena genetske povezanosti između stada svinja u Češkoj

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Rezime

Analizirani su proizvodni i reproduktivni podaci rase svinja češki landras. Stepen genetske povezanosti između zapata (mereno i izraženo kao ocena povezanosti; CR) je izračunata rešavanjem Animal model za jednu osobinu (STAM) i dve osobine (TTAM) - *Single trait* (STAM) and *two trait* (TTAM) *Animal model*. Prosečna CR za jedan zapat je izračunata kao prosečna povezanost sa svim ostalim zapatima izražena u procentima. Takođe je ocenjen CR unutar regiona Češke Republike. Genetska povezanost postoji u svih 51 analiziranih zapata. CR varira od 0,1% do 54,7% za STAM i od 0,1% do 56,0% za TTAM. Prosečna CR je bila 9,50% i 9,90% prilikom primene STAM i TTAM, respektivno. CR ispod 3% imalo je 17.6% zapata i ova vrednost nije bila pod uticalem korišćenog modela. Približno polovina analiziranih zapata je imala CR između 10 i 20%. S druge strane, samo 2,0% zapata (STAM) i 3,9% zapata (TTAM) je imalo CR iznad 20%. Primećena je visoka varijabilnost CR između regiona Češke. Najmanji CR je izmeren za zapate u regionu Zapadne Bohemije (4,4%). Suprotno, najviši CR je utvrđen za zapate u istočnoj Češkoj (11,9%) i Severnom Moravskom regionu (12,0%). Niske vrednosti uočene u poslednjim navedenim regionima verovatno su posledica niske proporcije zapata i relativno male količine životinja. Povezanost između zapata češkog landrasa je na dobrom nivou. Povećanje genetske povezanosti npr. korišćenjem većeg udela zajedničkih nerasta je i dalje potrebna.

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IMPACT OF VARIOUS FACTORS ON PROPERTIES OF FATTENING PIGS

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Abstract: The aim of this study was to determine whether the following traits of fattening pigs - slaughter age (SA), average life daily gain (ALDG) and daily weight gain of the carcass sides (DWGCS) were influenced by the following factors: sire breed, sire within the sire breed, genotype of fattening pigs, gender, gender within the sire breed, season of birth and gender within the genotype of fattening pigs. The research was conducted at the experimental farm and slaughterhouse of the Institute for Animal Husbandry, Belgrade-Zemun. The experiment included females and a male castrated animals. Sires of fattening pigs were pure breed: Swedish Landrace (SL, n = 10), Large White (LW, n = 3) and Pietrain (P, n = 3), while the offspring belonged to the following genotypes: Swedish Landrace (SL), and crossbreds: Large White × Swedish Landrace (LW x SL), Pietrain × Swedish Landrace (P × SL), [Pietrain × (Large White × Swedish Landrace)] P × (LW × SL), [Swedish Landrace × (Large White × Swedish Landrace)] SL × (LW × SL) and [Large White × (Large White × Swedish Landrace)] LW × (LW × SL). It was found that sire breed (SB), the sire within the sire breed Swedish Landrace (S:SL) and sire within the sire breed Pietrain (S:P) had very high statistically significant ($P<0.001$) influence on the variation of observed trait of fattening pigs. The sire within sire breed Large White (S:LW) had no influence on the variation of traits SA ($P> 0.05$). The pig gender (gender) in both models applied showed no statistically significant impact on the traits SA and DWGCS ($P>0.05$). Season of birth of fatteners (Season) had no effect on the variability of traits ($P>0.05$) except in a model 1 where it showed the effect on the properties of ALDG i DWGCS ($P<0.001$ and $P<0.01$). Gender within the sire breed (Gender: SB) had no statistically significant effect on the properties (P 0.05). Gender within genotype (Gender: Genotype) showed low statistically significant effect on the trait ALDG ($P <0.05$)

Key words: fattening pig, age, daily gain, warm carcass side

Introduction

Pigs are rare domestic animals that are bred only for one type of product - meat. Good feed conversion allows them enviable growth. In a relatively short period of time they are reaching optimal weight for slaughter which enables quick valorisation on the market. If rearing is organized in the period when the cheap food is available, i.e. expensive winter diet is skipped, the costs are much lower, and therefore the farm profit increased (*Paajenen, 2011*). Selection of boars should be careful and only the best individual animals must be chosen, particularly because they most affect the performance of the progeny of the farm. The effect of boars on the quality and characteristics of the pig population on the farm is great because a boar has a much larger number of offspring compared to single sow (*Čengić-Džomba et al., 2014)*. In Serbian agriculture, pig production is traditionally very important economic sector. That is why the intensification of swine production is the basic requirement and necessity in order to overcome the current lag, ensure the population's needs in food, stop imports and carry out preparations for the production of quality meat. The primary tasks in that respect is creating of highly productive breeds of pigs, winning of modern farming methods and the application of new technological solutions allowing for high production of the modern pig farms (*Jakić-Dimić et al., 2007*). Main objective in selection of boars for their further use in breeding should be their influence on improvement of economically important pig traits, i.e. production parameters in pig production. By artificial insemination, beside more rational use of boar, also greater effect in selection is achieved. Annually 2000 to 2500 doses of semen from single boar can be obtained, i.e. great number of offspring *(Radović et al., 2007b).* It is necessary to have knowledge of selected breed characteristics for the purpose of crossbreeding proper regulation of hybridization *(Johson, 1981).*

Production of pigs and pig meat depends on many factors: market and economic efficiency of production, the price of fattening pigs, methods of evaluation of fatteners, genetic and environmental factors (*Radović, 2012*). The meat production yield and quality of pigs depend on many factors: for example on breed, individual traits of pigs, feeding and housing conditions, the ability to adapt to different breed combination in the breeding and use of theirs genetic potential producing good quality, marketable commercial products *(Klimas and Klimienė 2001, Jukna et.al., 2003).* The main conditions for increase of economical production of pigs are: increasing the annual production of fattening pigs per sow, reduction of food consumption per kilogram of weight gain and higher meatiness of pigs (*Radović, 2012*). In the study of the average values of production traits of sons and daughters, *Petrović et al. (2002)* have established expressed effect of sires ($P<0,01$ and $P<0,05$) on age and average daily gain (from birth to the end of the test) with same average final body mass (103,0 kg). In studies of carcass quality of

five different genotypes (Swedish Landrace, F_1 crossbreds Swedish Landrace x Large White, and three-breed and four-breed combinations of crossbreeds between Large White, Swedish Landrace, Pietrain and German Landrace) with average pre-slaughter age of 181.3 days, *Gorjanc et al. (2003)* have found the average weight of warm carcass sides of 77.70 kg. At the same time, a significant effect ($P<0.05$) of genotype on all the characteristics of carcass quality was determined. There are a large number of studies on the impact of the sire breed, genotype and gender on the quality of pig carcass and meat quality of pigs, wherein the effect of sire breed or genotype and gender of offspring on carcass quality have been determined (*Edwards et al., 2003, Petrović et al., 2004, Jukna and Jukna, 2005, Petrović et al., 2006, Radović et al., 2008, Radović et al., 2009, Radović et al., 2010, Radović et al. 2012*). Boars also influence variation of pre-slaughter age. *Radović et al. (2003)* have found the impact of sires on the variation of pre-slaughter age of daughters. *Petrović et al. (2006)* have examined the influence of breed, sire and gender on the quality of pig carcasses grown on two farms (A and B). They have established the impact of sire genotype ($P<0.01$) on variation of all investigated traits of offspring on the farm B. However, on farm A the sire genotype had no impact ($P> 0.05$) on variation of daily weight gain of warm carcass sides.

Material and Methods

Investigations were carried out on the farm and experimental slaughterhouse of the Institute for Animal Husbandry, Belgrade-Zemun. The research included the offspring/progeny of three sire breeds (SL - Swedish Landrace; LW - Large white and P - Pietrain). The genotypes of parents and offspring are shown in Table 1. Dams tested offspring were SL (the largest breed in the herd, genotype 1) and F1 generation crosses (SL x LW, genotype 2). The sows were mated with boars of three breeds, so the resulting offspring were: pure breed (genotype 1); two breed crosses with a share of parental breeds 50:50 (genotype 2 and 5); two breed crosses with 75% of the sire breed (SL or LW, genotype 8 and 9) and three breed crosses (genotype 6).

Table 1. The genotypes of parents and offspring tested (finishing pigs)

Sire	Dam		Offspring(fatteners)	
Breed	Genotype	Mark	Genotype	Mark
SL	SL	1	SL x SL	1
	LWxSL	2	SL x (LWxSL)	8
LW	SL	1	LW x SL	2
	LWxSL	2	LW x (LWxSL)	9
P	SL	1	P x SL	5
	LWxSL	2	P x (LWxSL)	6

The study included 536 offspring of both genders (female and male castrated animals) originating from the 16 sires. There was 10 sires of SL breed (the sire numbers: 1, 2, 3, 7, 8, 9, 15, 16, 17 and 18), 3 sires of LW (the sire numbers: 4, 5 and 6) and 3 sires of P breed (the sire numbers: 14, 19 and 20). Minimum number of offspring per each sire was 9. Examined animals were born during the four seasons of the year (winter, spring, summer and fall), so that the fattening was also continuously performed. The fattening finished when offspring reached the body mass of about 100 kg; pigs were identified, weighed and shipped to the slaughterhouse of the Institute of Animal Husbandry.

Data analysis was performed using appropriate computer packages "LSMLMW and MIXMDL, PC 2 VERSION" (*Harvey, 1990*) and using the procedure of Least square method in order to determine the significance ($P<0.05$) of systematic influences on the properties of pre-slaughter age, the average lifetime daily gain and daily weight gain of the warm carcass sides. The models included: sire breed, sires within sire breed, season of birth, gender, gender within sire breed, genotype, and gender within genotype.

To test the phenotypic variability of age at the end of fattening, average lifetime daily gain and daily gain of warm carcass sides, two models were used.

Model 1.

$$Y_{ijklm}=\mu + R_i + O_{j:i} + P_k + P_{k:i} + S_l + \varepsilon_{ijklm}$$

where: Y_{ijklm} – expression of trait in m animal, of i boar breed, j sire within sire breed i , k gender and k gender within breed i, l season of birth; μ = general population average, R_i – sire breed effect (i=1, 2, 5); $O_{j:i}$ – effect of sires within the breed ($j{:}i_1$ =1, 2, 3, 7, 8, 9, 15, 16, 17, 18; $j{:}i_2$ =4, 5, 6; $j{:}i_3$=14, 19, 20); P_k – effect of gender (k =1,2); $P_{k:i}$ – effect of gender within the breed; S_l – effect of season of birth (l =1, 2, 3, 4); ε_{ijklm} – random error (residue).

Model 2.

$$Y_{ijkl}=\mu + G_i + P_j + P_{j:i} + S_k + \varepsilon_{ijkl}$$

where: Y_{ijkl} – expression of trait in l animal, of i genotype, j gender , k gender, j gender within genotype i, k season of birth; μ = general population average, G_i – effect of genotype (i =1, 2, 5, 6, 8, 9); P_j – effect of gender (j=1,2); $P_{j:i}$ – effect of gender within breed; S_k – effect of season of birth (k =1, 2, 3, 4); ε_{ijkl} – random error (residue).

Results and Discussion

All the traits tested have been adjusted to the same weight of warm carcass side (WWCS), which was 81.20 kg. The average values and standard deviations of adjusted traits are shown in Table 2. The studied genotypes of fattening pigs with average 204.91 days of age were 101.04 kg. From their birth to the end of the

fattening period, their average body weight increased by 488.88 g per day of life. Net daily gain of warm carcass sides, in average was 392.64 g/day of life.

Table 2. Means and standard deviations of growth traits of offspring

Trait		$\overline{X}$ ± SD
SA	Slaughter age, days	204,91 ±16,37
ALDG	Average lifetime gain, g	488,88 ±46,57
DWGCS	Daily weight gain of warm carcass side, g	392,64 ±57,87
MPK	Final body mass, kg	101,04±9,61

Table 3 shows the variability of growth traits of fattening pigs depending on the sire breed and sires within the breed. The average slaughter age of offspring of studied sires (Table 3) was 201.32 days. The youngest at slaughter were the offspring of Pietrain boars (194.57 days) and the oldest of Large White boars (209.15 days). The investigated trait varied between groups of half-siblings from different sires of Swedish Landrace (SL, $P<0.001$) and Pietrain (P, $P <0.001$) but not between the Large White sires (LW, $P> 0.05$) (Table 6). Test results show that the youngest and oldest fattening pigs at slaughter originated from the sire breed SL. In the study by *Petrović et al. (2006),* the age of the offspring of 203-204 days was determined, with a daily weight gain of warm carcass sides of 420-421 g, which are higher values than in our study.

The overall average for the lifetime daily weight gain was 515 g and varied under the influence of all the factors involved in model 1 ($P <0.001$ and $P <0.05$), with the exception of the factor Gender: SB where there was no effect. The offspring of boars P, were the youngest, because they had the most intense growth (578.47 g/day), which was by 81.86 and 108.54 g higher than fattening pigs whose sires were SL and LW breeds, respectively. Varying of ALDG in finishing pigs between different LW sires was statistically significant ($P <0.05$).

Table 3. The effect of sire breed and sires within the breed on slaughter age, lifetime gain and daily weight gain of the warm carcass side (LSMean ±S.E.)

Source of variation		SA[2)], days	ALDG, g	DWGCS, g
μ ± S.E.		201,32 ±1,52	515,00 ±3,47	419,49 ±5,10
SB[1)]	Sire number			
Swedish Landrace	1	210,78± 2,27	479,12±5,19	381,26±7,63
	2	203,45± 2,31	484,76±5,28	395,06±7,76
	3	202,42± 2,89	467,12±6,58	378,04±9,68
	7	192,32± 5,86	557,62±13,38	448,44±19,68
	8	177,02± 5,54	565,83±12,64	442,72±18,59
	9	194,79± 5,92	533,15±13,50	431,04±19,86
	15	203,84±5,49	472,60±12,52	383,90±18,41
	16	202,45± 5,09	482,59±11,62	390,19±17,09
	17	211,15± 5,05	466,85±11,52	378,69±16,94
	18	203,99± 4,70	456,50±10,72	367,28±15,76
	Average	200,22±2,04	496,61±4,65	399,66±6,84
Large White	4	210,12±2,26	462,68±5,15	362,94±7,58
	5	211,15±2,57	466,40±5,88	381,76±8,64
	6	206,18±2,39	480,72±5,45	393,41±8,02
	Average	209,15±1,74	469,93±3,97	379,37±5,84
Pietrain	14	208,98± 3,25	467,73±7,42	388,51±10,92
	19	179,61± 6,11	615,15±13,93	506,29±20,49
	20	195,13± 5,39	652,51±12,29	543,49±18,08
	Average	194,57 ± 3,49	578,47±7,96	479,43 ±11,70

[1)]SB-sire breed; [2)]SA- slaughter age, ALDG-average lifetime daily gain, DWGCS- daily weight gain of warm carcass side

The mass of warm carcass sides of all tested fattening pigs increased by an average of 419.49 g/day of life. The investigated traits varied under the influence of the sire breed ($P < 0.001$), sires within SL ($P < 0.001$), LW ($P < 0.01$), P ($P < 0.001$), season of birth of pigs ($P < 0.01$) but not under the influence of gender of fatteners ($P > 0.05$). Mean DWGCS values per sire breeds were as follows: 399.66 (SL), 379.37 (LW) and 479.43 g (P).

Table 4 shows the average values for the studied traits of fattening pigs under the influence of gender and season of birth.

Table 4. Average values (LSmean ± SE) slaughter age, lifetime daily gain and daily weight gain of the warm carcass side under the influence of gender and season of birth (model 1)

Source of variation		SA[2)], days	ALDG, g	DWGCS, g
Gender	M[1)]	200,30±1,70	522,65± 3,89	423,98±5,72
	F	202,34±1,83	507,36± 4,17	415,00±6,14
Season	Winter	199,66±4,20	529,50± 9,59	436,34 ±14,10
	Spring	207,49±2,48	473,18± 5,67	383,05±8,33
	Summer	199,24±2,46	529,10± 5,61	432,60±8,26
	Fall	198,87±2,32	528,23± 5,29	425,96±7,78

[1)] M- male castrated animals, F –females;

[2)] SA- slaughter age, ALDG-average lifetime daily gain, DWGCS- daily weight gain of warm carcass side

In regard to gender (Table 4), we see that male castrated animals, in comparison to females, finished fattening two days earlier and had higher lifetime gain and a higher daily weight gain of the warm carcass side. The difference in LSM values between ALDG of castrated males and females was statistically highly significant (P <0.001). The lowest gains were recorded in animals born in the spring season (473.18 and 383.05 g). Variations in ALDG and DWGCS were lower between other seasons of birth of investigated pigs. Season of birth influenced the variation ALDG (P <0.001) and DWGCS (P <0.001) using Model1. *Gogić et al. (2012)* have concluded that age of animals was 191 days, i.e. by 11 to 15 days less compared to this research. The same group of authors has established statistically significant effect of sire breed, sire within Swedish Landrace and Pietrain on the traits of offspring which is in line with our research.

The results of the study of phenotypic variation of pre-slaughter age and gain of offspring per genotype, gender and season of birth are shown in Table 5. Using two models for analyzing the above mentioned traits, it can be concluded that the trait SA did not vary significantly (P> 0.05) between genotypes, genders and season of birth of fatteners. Traits ALDG and DWGCS varied between genotypes of pigs (P <0.001). The highest ALDG and DWGCS were recorded in fatteners of genotype 6. Genotype 6 were three breed crosses whose sires were Pietrain [Px (LWxSL)]. The pigs of breed SL (genotype 1) realized lower average value of ALDG and DWGCS than the general average. Two breed crosses P x SL (genotype 5) and LW x (LWxSL) (genotype 9) had ALDG less than 480 g. Also, they had a slower increase in the weight of warm carcass sides compared to other genotypes of pigs.

ALDG values in castrated male pigs were higher than in females (P <0.01). This trait varied (P <0.05) between the genders within the genotype of pigs. The biggest difference between the mean values of ALDG was between males and females of genotype 6 (57.1g).

Table 5. The effect of genotype, gender and season (model 2) on growth traits (LSMean ±S.E.)

Source of variation		SA[2)], days	ALDG, g	DWGCS, g
μ ± S.E.		204,94 ±1,32	494,86 ±3,52	403,45 ±4,54
Genotype	1[1)]	203,06±1,32	487,15±3,51	392,69±4,52
	2	206,25±1,66	483,02±4,42	389,16±5,70
	5	210,06±4,61	466,93±12,29	387,21±15,83
	6	201,00±3,08	550,84±8,20	459,31±10,57
	8	199,82±3,04	503,59±8,11	412,50±10,44
	9	209,46±2,94	477,65±7,83	379,83±10,09
Gender	M[2)]	203,49±1,80	504,15±4,80	407,97±6,19
	F	206,39±1,68	485,57±4,48	398,93±5,77
Season	Winter	201,93±3,65	504,80±9,73	421,61±12,53
	Spring	204,26±2,00	484,68±5,33	391,03±6,86
	Summer	208,85±1,72	495,20±4,58	401,62±5,90
	Fall	204,73±1,43	494,78±3,81	399,53±4,91

[1)]1-SL, 2- LWxSL, 5-PxSL, 6- Px(LWxSL), 8- SLx(LWxSL), 9- LWx(LWxSL); [2)] M- male castrated animals, F –females; [3)] SA- slaughter age, ALDG-average lifetime daily gain, DWGCS- daily weight gain of warm carcass side

Gogić et al. (2013) found that the value of lifetime daily gain for females was 485 g, which is in agreement with this research (model 2).

Table 6. Statistical significance (level of significance) of effects included in the models (1 and 2) when analyzing the properties SA, ALDG and DWGCS

Source of variation (effect)[1)]		SA[2)]	ALDG	DWGCS
Model 1	SB	**[3)]	***	***
	S:SL	***	***	***
	S:LW	NS	*	**
	S:P	***	***	***
	Gender	NS	***	NS
	Season	NS	***	**
	Pol:SB	NS	NS	NS
	R^2	0,144	0,449	0,228
Model 2	Genotype	NS	***	***
	Gender	NS	**	NS
	Season	NS	NS	NS
	Gender:Genotype	NS	*	NS
	R^2	0,052	0,168	0,106

[1)]SB-sire breed, S:SL-sires within Swedish Landrace breed, S:LW-sires within Large White breed, S:P- sires within Pietrain breed, Gender:SB-gender of offspring within sire breed, Gender:Genotype-gender of offspring within genotype; [2)] SA- slaughter age, ALDG-average lifetime daily gain, DWGCS- daily weight gain of warm carcass side; [3)] NS=P>0,05; *=P<0,05; **=P<0,01; ***=P<0,001

From Table 5 (Model 1) we can see that the sire breed, sires within breed SL and breed Pietrain influenced statistically highly (P <0.001) variation of all traits of offspring. No variation of any of the studied properties (P> 0.05) influenced by factor Gender: SB was determined. Season of birth of offspring significantly influenced the properties of ALDG and DWGCS only in model 1 (P <0.001 and P <0.01). The differences in the mean values for the property ALDG between females and male castrated animals were statistically highly significant (P <0.001 and P <0.01) in both models used. The values of the coefficient of determination for Model 1, ranged from 0.144 (SA) to 0.449 (ALDG). The coefficient of determination R^2 shows that the effects included in the Model 1 (sire breed, sires within the breed, gender, season and season within sire breed) explained 14.4% of the variation of SA, 44.9% of the variation of ALDG, and 22.8% of the variation of DWGCS. The coefficient of determination R^2 shows that the effects included in the Model 2 (genotype, gender, season and gender within the genotype) explain 5.2% of the variation of SA, 16.8% of the variation of ALDG, and 10.6% of the variation of DWGCS. So, variation of the trait ALDG in both applied models is best explained as the result of the effect of most factors, while the variation of SA trait in both models is least explained as the result of the effect of factors. The gender of offspring from the model 1 only affected the trait of lifetime gain, in accordance with the research by *Petrović et al. (2006),* but with no influence on the trait SA, which is contrary to the results of the same group of authors. Sire breed significantly affects the investigated traits of offspring (*Pušić and Petrović, 2004; Radović et al., 2007a; Gogić et al., 2012*). Season of birth and animal genotype influence the properties (*Radović et al., 2003; Radović et al., 2007a*).

Conclusion

Based on the results obtaine din the present study, it was found that sire breed, sire within breed SL, and sire within the breed P, highly significantly affected all studied traits of pigs (P <0.001). Sires within LW did not affect only SA. Gender within sire breed (model 1) and season of birth of pigs (model 2) had no effect on the expression of traits of pigs (P >0.05), while season of birth influenced the ALDG and DWGCS traits of finishing pigs and (model 1). Gender of fatteners and gender within the genotype influenced only ALDG. Male castrated animals had higher values for properties ALDG and DWGCS compared to females. Animals born in the spring had the lowest gain, while the animals was born in the winter had the best values for growth traits. The best values for gain were recorded in fatteners Px (LWxSL), while the lowest value for the trait DWGCS was recorded in animals of genotype LWx (LWxSL). The highest gains were recorded

in offspring of sires of breed Pietrain while the lowest values in offspring of sires of Large White breed.

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Uticaj različitih faktora na osobine tovljenika svinja

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Rezime

Cilj ovog istraživanja je da se utvrdi da li su osobine tovljenika uzrast pri klanju (SA), prosečan životni dnevni prirast (ALDG) i dnevni prirast mase tople polutke (DWGCS) bile pod uticajem sledećih faktora: rasa oca, otac unutar rase oca, genotip tovljenika, pol tovljenika, pol unutar rase oca, sezona rođenja tovljenika i pol unutar genotipa. Istraživanje je obavljeno na farmi i eksperimentalnoj klanici Instituta za stočarstvo, Zemun-Beograd. Ogledom su obuhvaćena ženska grla i muška kastrirana grla. Očevi tovljenika pripadaju čistim rasama: švedski landras, veliki jorkšir i pijetren, dok potomci pripadaju sledećim genotipovima: švedski landras (SL), i melezi: veliki jorkšir×švedski landras (LW×SL), pijetren×švedski landras (P×SL), [pijetren×(veliki jorkšir×švedski landras)] P×(LW×SL), [švedski landras×(veliki jorkšir×švedski landras)] SL×(LW×SL) i [veliki jorkšir×(veliki jorkšir×švedski landras)] LW×(LW×SL). Utvrđeno je da rasa oca (RO), otac unutar rase oca švedski landras (O:SL) i otac unutar rase oca pijetren (O:P) utiče na variranje ispitivanih osobina tovljenika veoma visoko statistički značajno ($P<0.001$). Otac unutar rase oca veliki jorkšir (O:LW) nema uticaja na variranje osobine SA ($P>0.05$). Pol tovljenika (Pol) u oba primenjena modela nema statistički značajnog uticaja na oobine SA i DWGCS ($P>0.05$). Sezona rođenja tovljenika (Sezona) ne utiče na variranje osobina ($P>0.05$) osim u modelu 1 gde pokazuje uticaj na osobine ALDG i DWGCS ($P<0.001$ i $P<0.01$). Pol unutar rase oca (Pol:RO) nema stitistički značajnog uticaja na osobine ($P>0.05$). Pol unutar genotipa (Pol:Genotip) pokazuje nizak statistički značajan uticaj na osobinu ALDG ($P<0.05$).

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DISEASES OF SUCKLING PIGLETS: HEALTH AND REPRODUCTION PROBLEMS ON COMMERCIAL FARM

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Review paper

Abstract: Good health is a prerequisite for good pig reproduction and profitable production. Diseases of suckling piglets, weaned pigs and finishing pigs might endanger production. Diseases of pigs that may be present on commercial farms can be controlled by use of prophylactic and therapeutic measures to keep them under control. Particularly sensitive and critical periods are dusting, the first 24-48 hours after farrowing, from 7 to 10 days after birth, and the period from 10 to 14 days after piglets are weaned. It is possible by using biotehnical rate to put emphasis on preventing the diseases of swine and to improve production.

Key words: suckling piglets, health, reproduction, swine

The most common health and reproductive health problems on a commercial pig farm

In intensive pig production control of herd reproduction is the primary task. It is known that in comparison to other types of domestic animals, pigs are characterized by very high reproductive potential, considering that they sexually mature early, have high ovulation rate, the relatively short period of gestation and lactation, fast establishment of gestation after weaning the previous litter. From the economic point of view, proper, regular reproductive activity of pigs is of a great importance. If one herd reproductive efficiency is satisfactory, it is usually estimated on the basis of: age at which gilts farrow for the first time, the length of their reproductive exploitation, the duration of the interval between individual farrowing rate and litter size in weaning. Reproductive activity in pigs is influenced by many factors, among which are very important: hereditary factors, endogenous

factors (hormones, immune-globulins,enzymes), the influence of the environment, the presence of pathogens, as well as management and production technology *(Uzelac and Vasiljević, 2011)*. These additional factors also affect the reproductive efficiency: the weight gain, nutrition, season, farm location, microclimate, implementing biosecurity measures, herd size, herd health status (presence of breeding, parasitic and infectious diseases), body condition and the method of application of artificial insemination *(Lončarević et al., 1997, Petrujkić et al. 2011)*. The problem of infertility is a common problem on commercial farms. The causes of infertility are various and numerous. Current problem of most of our farms is the emergence of seasonal infertility that is present during the summer months and is a serious impediment to producers who want to maximize their reproductive efficiency of the herd *(Petrujkić et al., 2011)*. According to presented, in intensive pig production much attention is now paid on obtained optimum of microclimate conditions in facilities for housing, using computerized systems for ventilation, cooling, lighting, water, feeding, manure with programmable desired parameters at specific time intervals, thus creating animals the most favorable conditions to maximally express their genetic potential, achieve high productivity and greatly reduce the stress. Adequate health care for farm animals, a high level of hygiene of animals, equipment and people as well as the precise application of all procedures in the technology of artificial insemination are the primary requirements for high reproductive efficiency of breeding animals *(Stančić et al. 2012)*.

Conventional assessment of the quality of semen, as a segment of technology of artificial insemination, is practiced largely on our commercial farms. Classical assessment of the semen in commercial terms can be used to recognize ejaculates with low potential for fertilization, but does not achieve high efficacy in predicting fertility parameters in the field *(Tsakmakidis, 2011)*. Therefore, in order to combat infertility and to control reproductive efficiency of pigs today in cooperation with the Institute laboratory methods such as motion estimation using computer analysis (CASA), the automatic analysis of sperm morphology (ASMA), determination of the integrity of chromatin via flow cytometry, HOS test, etc. are implemented successfully. In this way, fertility of boars can be continuously monitored and it enables promptly reaction to the immediate production. Technology preparations of heterosperm insemination doses involving sperm of two or more terminal boar breeds is also used in artificial insemination on our commercial farms to produce more piglets per sow *(Vasiljević, 2012)*.

The use of deep frozen semen is also used in the world on industrial swine farms. The advantage of deep frozen semen is that it keeps the genetic material for a longer time period and significantly reduces the risk of introduction of the disease in the herd *(Stankovic et al., 2007)*. However, the deep cryopreservation did not

enter into wide practice because technologies of deep freezing have not been developped in a satisfactory extent and a there is a low percentage of pregnancy and litter size *(Vidović et al., 2011)*. The phenomenon of stress is also one of the serious problems in commercial farms. Farms that are still developing their management have a greater problems with stress than farms that have organized complete production. The requirements of modern pig production today are reduced stress to the minimum and provide maximum comfort of animals (welfare). Regarding this it is important to know and consider mechanisms for adaptation syndrome and stress reactions and to provide to the animals more adequate living conditions in order to expect productivity on desired level. High levels of corticosteroids in the blood of animals exposed to stress are acting to reduce their resistance and thus allowing them to become highly susceptible to various infections. Therefore, it is very important to promote animal welfare in farm conditions through the development and improvement of man's consciousness in the direction of respect, caring and responsibility towards animals, as well as the application of technical and technological solutions in the production of animals that will provide maximum comfort and convenience.

Technology of feeding of farm animals also plays a significant role in the prevention of stress and is also a very important factor maintaining high health and reproductive status. Too much fattened sows as they carry a large number of offspring, and in addition consume a large amount of food in facilities where the increased humidity and temperature exist, are more susceptible to stress and show signs of respiratory distress. It's one of the reasons for the introduction of recommendations related to diet by production stages and categories of animals. Precisely is defined, for example the feeding curve of breeding sows at each stage of production in order to facilitate the early entry into estrus after weaning of piglets, a large number of super ovulated and implanted embryos, a greater number of live, vital piglets, the greater the amount of the produced milk during lactation and that at the same time is possible to preserve the fitness and health of cows in order to live longer and have longer productive life and to lower the use of medications. Thanks to this approach, today is nothing new that we have commercial farms with 35 and more weaned piglets per sow per year.

Production of pigs on commercial farms is largely burdened by diseases of piglets. Pathology of piglets is a very dynamic discipline within the entire herd epizootiology in which the large agglomerations of animals in a confined space can easily come up with horizontal and vertical transmission of infection and certain microorganisms due to the forced cultivation and possession of lead to the production and technological disease. Great importance is attached to the variations of pathogens in pigs, not only in showing resistance to drugs, but the occurrence of genetic recombination, which affect the clinical picture and course of the disease,

all of which makes it difficult to diagnose and apply therapy and prophylaxis *(Blackburn, 1995, Bojkovski et al., 1997, 2005).* In our pig farms most fequently we encounter: Neonatal colibacillosis, endemic disease, necrotic enteritis, circoviral infections, colitis caused by spirochetes, enterohaemorrhagic syndrome, dysentery and respiratory disease complex. In recent years, in the world and on our pig farms there was a mass occurrence of respiratory disease complex (PRDC), which is becoming a serious health problem in all technological stages of production. The complex is a respiratory disease of pigs characterized by simultaneous infection of lung tissue with more respiratory pathogens and is a common term for pneumonia in pigs with multifactorial etiology. Isolated pathogens vary between and within production herds *(Honnold, 1999; Ivetić et al., 2005; Golinar et al. 2006).* Control of respiratory disease complex is difficult and complicated. The significance of respiratory diseases complex is based on the interaction of respiratory pathogens. Knowledge of the interaction of respiratory pathogens should be considered in order to implement effective control measures.

Respiratory disease of pigs occur if the causative agents are present in the same habitat or if due to an unknown cause for us, the immune response mechanisms of respiratory system weakens *(Ivetić et al. 2005)*. Unlike classical spreading control of infectious diseases of pigs that persist in our country whose fighting is a legal obligation, a detection and suppression of technopathies are more an economic need of the producers themselves.

Disease of suckling piglets

In suckling piglets great importance is given to variations of pathogenic microorganisms, not only in the expression of resistance to drugs, but the occurrence of genetic recombination, affecting the clinical picture and course of the disease, all of which complicates the diagnosis, therapy and prophylaxis *(Blackburn, 1995, Bojkovski and sar.1997, 2005, Gagrčin et.al. 2002,Šamanc, 2009).*

Neonatal colibaciosis

Neonatal colibacillosis is common in farrowing facilities. The receptive piglets are infected with enterotoxigenic strains of *E. coli* that are capable of adhesion to intestinal epithelium (by means of one or more fimbrial adhesin (F4, F5 or F41), production and secretion of enterotoxines. Enterotoxines have the ability to inhibit certain enzymes, induce the creation of PGF2α prostaglandins which stimulate the smooth musculature movements, then stimulates adenyl cyclase which is a consequence of the conversion of the adenyl-triphosphate in adenyl monophosphate (cAMP), it causes the secretion of an active water and electrolytes into the lumen of the intestine. The colon is not capable for

reapsorbance of such a large amount of intestinal liquid. Dehydration or loss of body weight and slower progression of piglets requires rehydration which can be carried out either oral or intraperitoneal route and application antimicrobial treatment according to the microbiological resistance pattern analysis *(Bojkovski et al. 2008a, Šamanc, 2009).*

Neonatal diarrhoea of piglets aged 1 to 6 days is due to ETEC (enterotoxigenic *E. coli*) belonging to the groups 08, 09, 064, 0101, 0149, 0157 which produce one or more enterotoxins Sta, Stb or Lt. ETEC isolated from suckling piglets with diarrhoea between 14 and 21 days of age often belong to serovars 08, 0115, 0147 and produce Stb Lt enterotoxin. Disease severity depends on pathogenic strains of *E. coli* and conditions that increase the sensitivity of animals (the absence of specific antibodies or other protective substances important for immunity). Accordingly, any factor that hinders the introduction of colostrums antibodies in the first hours post partum is a predisposing factor for disease. Of particular significance is the nutrition of pregnant sows, preparation of sows for farrowing, the occurrence of hypogalactia and agalactia, size and vitality of newborn piglets and bad zoo hygienic conditions. With birth before reaching the antibodies in colostrum a piglet encounter contaminated surrounding in the farrowing pits and on the skin of the udder resulting in infection of germs mainly from the intestinal flora of sows. When the piglets are infected by considerable number of pathogenic strains of *E. coli* colibacillosis occurs. Contributing factor is the relatively high pH in the stomach of piglets which enables easy colonization of bacteria in the proximal small intestine parties. Neonatal colibacillosis is manifested as diarrhoea with pale-yellow colour which often looks foamy. Piglets are apathic and dehydrated. There are cases when whole litter may be lost. The body temperature is at the lower border of physiological value and often is subnormal. Only in a case of septicaemia body temperature is elevated. In peracute cases of the disease autopsy report are negative. Diagnosis is based on isolation of bacteria from the abdominal organs. In the case of acute disease piglets are dehydrated and perineal region is tainted by excrements. The stomach is moderately dilated. In some cases, subserosal and submucosal petechial haemorrhage can be found. Some segments of the intestine may be full of air content *(Bojkovski et.al 2008b, 2010a,b,c,d, 2011b).*

Escherichia coli diarrhoea occurs usually in suckling piglets in the first few days of life, but it can also occur after weaning with occasional septicaemia. Colibacillosis occurs in all countries practicing intensive pig farming. This disease is recognized 50 years ago. The treatment is then carried out empirically. Recurrence of diseases with an impact on other animal species and to humans, led to intensive colibacillosis research advances in molecular biology, simplified the identification of this pathogen virulence factors.

Pathogenic *E. coli* are easily isolated and morphologically different from bacteria with Gram negative appearance. Most of the pathogenic strains intend to form smooth mucoidal colonies, whereby some of those are β haemolytic *(Šamanc, 2009)*. Virulence factors include fimbriae (pilli), exotoxins (enterotoxin), endotoxin and capsule. Fimbriae are specific protrusion tread of bacterial cells that allow bonding to specific receptors on the surface of the enterocytes in the small intestine mucosa. This process is also called the colonization.

Pathogenic strains secrete one or more exotoxins (enterotoxin in pathological sense) having a local and/or systemic action. This group included strains is called enterotoxigenic *E. coli* (ETEC). The most common ETEC identified in 5 different types of files that are antigenically different from each other: F4 (K88), F5 (K99), F41, F6 (987p) and F18. First 4 types mediate the adhesion of ETEC for neonatal piglet enterocytes, while F18 and F4 partly mediate colibacillosis of weaned pigs. Certain strains may cause erosion of the mucous membrane and are referred to as an attaching and effacing *E. coli* (AEEC).

Toxins produced by ETEC are labile toxin (LT), stable toxin A (StA), stable toxin B (StB) and vrotoxin (Shiga-like toxin, SLT). These three respectfully cause hyper secretion of fluid in the guts and causes verotoxin vascular lesion which is oedema disease basis. To conclude we can say that the pigs and the surrounding area introduce many different strains and types of *E. coli* to newborn piglet *(Šamanc, 2009)*.

Control

Empirically, colibacillosis is the disease of facilities and technology of growing animals. Preventive measures, that are definitely recommended, include the purchase or production of semen for artificial insemination from trusted boars with no history of colibacillosis. Sows should be introduced to housing 3-6 weeks before insemination or before farrowing to develop immunity to the pathogen that are present locally in order to enable production of sufficient quantities of the specific antibodies in the colostrum and milk.

Preferably, immunity of sows is induced by vaccination against endogenous, locally isolated *E. coli* or bacterial vaccine based on a saw, toxoid or anavaccine. Pregnant sows are vaccinated twice at an interval of 2-3 weeks before farrowing. A recent assessment of the value of the vaccine and vaccination in the United States (National Animal Health Monitoring System) showed that it is the most favourable (cost-effective) procedure for producers.

The historically present method of sows' immunisation is giving waste from farrowing facility to pregnant sows during the last third of pregnancy. Waste includes pathogens that are found in the farrowing pen and stimulates the production of antibodies for future lactation. Oral vaccination of sows with virulent strains in milk is a method that was shown successful before the start of the modern

era of vaccination. System all in / all out for growing pigs is definitely recommended. Fumigation should be done in the house which is thoroughly cleaned, disinfected and dried after roasting previous farrowing. The build-up of pathogens is regulated by strict farm sanitation program. The farrowing pen should be designed to provide a dry, comfort midfield for mothers and piglets, with the optimum temperature (about 32^{o}C) with less stress. If there is a disease incidence history on the farm the veterinary service has to be ready for immediate treatment of patients. Antimicrobial drugs can be administered orally or by injection. Due to the potential spread of pathogens, all piglets in a litter (including those without symptoms) should be under the same treatment. To suckling piglets antibiotics are given through water. Results of susceptibility testing are adequate executive information when choosing an antimicrobial agent. Oral rehidratation solutions are used to prevent terminal loss of electrolytes. Various products have proven to be good supportive therapy: plasma proteins, zinc oxide, organic acids and probiotics. Considering that some breeding lines of pigs are resistant to certain pathogenic *E. coli* choosing these lines in breeding easier to control colibacillosis.

Necrotic enteritiris of pigllets

The first few days after farrowing, have the greatest potential for necrotic enteritis occurrence in piglets. Rarely occurs in pigs at the age of 2 to 4 weeks. Necrotic enteritis have a wide range of morbidity and mortality. The causative agent is a Gram-positive, anaerobic, sporulating bacteria: *Clostridium perfringens* type C with α and β toxins. *Clostridium perfringens* could be found in five toxogenic types (A, B, C, D, E) are distinguished by major toxins they produce (alpha, beta, epsilon, or iota); all types produce alpha toxins. It is known toxin β is paralytic in the jejunum and ileum, also necrotizing beta toxin causes the severe necrotizing in bowels. Disease associated with *Clostridium perfringens* and other clostridia occur in many different animal species. In swine, three rather common diseases occur. Enterotoxemia associated with *C. perfringens* type C is characterized by variable morbidity and high mortality in neonates or suckling pigs with sudden deaths, bloody diarrhoea, or necrotic enteritis. Neonatal diarrhoea is also associated with *Clostridium perfringens* type A but with moderate morbidity and lower mortality compared to *Clostridium perfringens* type C. *Clostridium difficile* infection of the cecum and colon produces diarrhoea with variable morbidity and mortality. Virulent strains of *Clostridium difficile* are known to produce an enterotoxin and a cytotoxin that are associated with both systemic illness in pigs and localized typhlocolitis and oedema of mesocolon.

The highest concentration of pathogic bacteria and product were determined in jejunum, so it seems to be the reason that the macroscopic changes in the most intense form are found in this segment of the intestine. Necrotic-hemorrhagic enteritis and body fluid in the abdominal cavity are visible.

Mesenteric lymph nodes are congested. The bowel wall shows haemorrhage and coagulation necrosis affecting the mucosa, crypts and Payer's intestinal patch.

Transmission of the disease is the direct or indirect: by faecal-oral route pathogen is transmitted from sow to piglet.The spores remain infectious for at least a year in untreated farms. Poorly chlorinated water is risk factor. A common cause of infection is contaminated water. Indirectly the cause is transferred via formities.

The toxin is found in bowels, peritoneal fluid, and can cause the encaphalomalacia, adrenal cortical necrosis, nephrosis and pulmonary oedema *(Bojkovski et.al.2011a).*

Control

In order to control this disease several different strategies are used. Effective but expensive is application of antitoxin to newborn piglets on the first day of life, which Iowa State University recommend within two hours of birth. Preventive therapy is based on penicillin preparations given in the first day of life with good hygiene farrowing pens. It is possible to prevent disorders caused by toxins vaccination of sows twice (2 and 1 months prior to farrowing) to raise the level of inmunoglobulines in colostrum. Where control is less than satisfactory, an autogenous vaccine is prepared from an isolate from a piglet with typical disease course and it may be used to vaccinate the sows. Some benefit may be obtained by feeding antibiotics with anticlostridial activity to sows before farrowing and during lactation. Antimicrobial agents used include bacitracin, tylosin, lincomycin and others.

Transmisible gastroenteritis(TGE)

Transmissible gastroenteritis is a highly contagious disease of Corona viral etiology. The virus multiplies in the epithelial cells of the small intestine, followed by a short incubation with high morbidity in all categories and almost 100 percent mortality in suckling piglets in the first seven days of age. This mortality is explained in the time required for regeneration of the intestinal epithelium, since in suckling piglets this takes between 8 and 12 days, and in adults of between 3 and 4 days.

The virus enters the cylindrical intestinal cells of the small intestine. Epithelial renewal is not impaired. The acidic environment of the stomach is the first barrier but virus overcomes it is stable at pH 3. The infection of epithelial cells the virus achieves his second feature, and it is resistant to the action of trypsin.

The incubation period is very short from 18 hours to 3 days. The disease is characterized by a yellow-green or green diarrhoea, vomiting, rapid dehydration and a number of animals, hipogalactia or agalactia and abstinence from food in lactating sows and the highest mortality of the youngest category. Body temperatures are normal or slightly elevated. In fattening pigs, the watery faeces, and body weight losses ranging from 4-5 and up to 10 kg per individual. The

illness usually lasts 3-7 days after adult animals back body condition. The piglets that survive TGE remain undeveloped *(Šamanc, 2009).*

When macroscopic examination of dead animals stomach and small intestine are almost empty, the tympanic and transparent. In some cases, the stomach is filled with milk clotting. Transmissible gastroenteritis (TGE) only occurs in pigs. In pigs that never had contact with the germ, all age categories are susceptible. TGE occurs throughout the year but is slightly more common in the cold weather. Occurs in all countries where pigs are bread, but is relatively rare in Europe.

Corona virus is etiological agent (TGEV). The isolates were mainly enteropathogenic. The virus is unstable at 22 °C, but well preserved if frozen. Canine corona virus and feline infectious peritonitis are similar. Variant of the intestinal viruses has affinity for the lung tissue. This variant infects epithelial cells of the respiratory system and alveolar macrophage and the infection is referred to as PRCV (porcine respiratory corona virus). PRCV was obtained from TGEV deletion of the S-gene having lost a tropism for enterocytes, but with enhanced tropism towards the lungs. Serological TGEV and PRCV give cross-reactions, but there are tests that distinguish them. TGEV sensitive to a large number of disinfectants: iodine, quaternary ammonium salts, phenol and sodium hypochlorite.

A pig that has overcome the TGE developed antibodies but continue to excrete virus in faeces and nasal secretions next 2-8 weeks, certain individuals intermittently secrete the virus a year and a half ago. TGE virus was isolated from the lung and intestine, and after three and a half months post mortem. Infected sows milk secreted virus to suckling. A faeces is the main source of infection, but the virus is probably transmitted, airborne, for a small distance. Other animals such as dogs, cats, foxes and birds can transmit the virus to different distances and variable timeframe. Farm equipment is an important source of infection. If one went to the farm virus remains longer period of time, especially in the winter month

Control

Preventive measures for negative herds include maintaining a closed herd and implementing strict biosecurity practices. Necessary acquisitions should be from herds that have no recent history of TGE. Additions should be serologically negative before and after a quarantine period of 30 days. The all in/all out system of production with cleaning and disinfection between farrowing is helpful. There are attenuated and killed virus vaccines. Vaccines usually stimulate immunity in the dams. Vaccines are usually administered to dams at several intervals prior to farrowing. In acute outbreaks, pregnant sows and gilts that are two or more weeks prior to farrowing can be induced with faeces or piglet intestinal homogenates from

the farrowing house. Antibodies in their colostrum and milk will then provide protection for the most critical time for their piglets.

Piglets less than three weeks old with TGE seldom respond to treatment. Treatment include weaning, oral electrolytes, and a warm environment. Older swine usually recover spontaneously. Piglets at least one month old before onset usually recover if provided with a nutritious starter feed, warm housing and good care. Antibiotics added to feed or water might be of value in preventing secondary bacterial infections.

Porcine epidemic diarrohea (PED)

Corona virus causes porcine epidemic diarrhoea. The virus is unrelated to porcine corona viruses that cause TGE, porcine respiratory corona virus (PRCV) and hemagglutinating encephalomyelitis virus (HEV). It has some antigenic determinants in common with feline infectious peritonitis virus.

Corona virus is disseminated in the faeces of infected pigs for at least seven days post-inoculation. Fomites and vehicles can also spread virus indirectly. On sites where PED is endemic and there is frequent or continuous farrowing, virus is maintained in successive generations of susceptible piglets.

The corona virus of PED, like that of TGE, is in feces and usually is transmitted orally. It replicates primarily in enterocytes on villi of the small intestine, and to a lesser degree in cryptal cells of both small intestine and colon, causing degeneration and necrosis of enterocytes. Many enterocytes are destroyed and replaced by cuboidal or flat epithelial cells. Intestinal lesions of PED are similar to those of TGE but develop less rapidly and are less severe.

Control

Pregnant sows can be intentionally exposed more than two weeks prior from parturition is the disease is present. Sanitary and quarantine measures may slow the spread of PED. There is no effective treatment other than good care.

Conclusion

The combat with different pathogens which are present in industrial swine farms has basis in the implementation of good management practice and the introduction of new breeding systems.

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Bolesti prasadi na sisi: zdravstveni i reporoduktivni problemi na komercijalnoj farmi

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Rezime

Dobro zdravlje svinja je uslov dobre reprodukcije, odnosno rentabilne proizvodnje. Bolesti prasadi na sisi, odbijene prasadi i bolesti tovnih životinja mogu da ugroze proizvodnju. Oboljenja svinja koja mogu da budu prisutna na komercijalnim farmama moguće je primenom profilaktičkih i terapeutskih mera držati pod kontrolom. Posebno osetljivi kritični periodi su samo prašenje, prvih 24-48 časova posle prašenja, od 7 do 10 dana nakon prašenja i period 10 do 14 dana po zalučenju prasadi. Moguće je primenom biotehnčkih mera, stavljanjem akcenta na preveniranje bolesti svinja unaprediti proizvodnju.

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CONTROL AND DIAGNOSTICS OF PORCINE RESPIRATORY DISEASE CAUSED BY *ACTINOBACILLUS PLEUROPNEUMONIAE*

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Abstract: Actinobacillus pleuropneumoniae (App) is the etiologic agent of porcine pleuropneumonia and one among the most important bacterial pulmonary pathogens in pigs. This aim of research was to establish the diagnosis of respiratory disease detected on a pig farm and implement appropriate control measures. The research methods included clinical and gross pathological examination of diseased and dead pigs, and control of respiratory organs at the slaughterhouse. Lungs, tonsils and lymph node samples were obtained from pigs at slaughter for bacteriology isolation and blood samples were taken for detection of specific antibodies against App. By examination of respiratory organs of 360 fatteners at the slaughter line, no visible changes in lung tissue were observed only in 19,44% of examined pigs. In others, examination of respiratory organs revealed pathological changes indicative for App (pleuritis, *Pleuropneumonia haemorrhagica necroticans*). Serological examination revealed presence of specific antibodies against App in 50% of sera samples (ELISA test). By bacteriological examination the following bacteria was detected: *Haemophilus parasuis*, *Pasteurella multocida, Trueperella pyogenes, Staphylococcus aureus, Mannheimia haemolytica*. The App was detected in only 2 examined samples. Results of present study confirm that the control at the slaughterhouse and serology testing can be a useful tool for the detection of pigs infected with App.

Key words: porcine respiratory disease, *Actinobaciluus pleuropneumoniae*

Introduction

Respiratory diseases of swine are among the most important problems causing huge economic losses to the swine industry worldwide *(Došen et al., 2014a; Gottschalk, 2012)*. Intensifying of swine industry increases the frequency

and economic importance of this disease regardless of the applied measures for their suppression and eradication *(Prodanov-Radulović, 2015)*. Occurrence of swine respiratory diseases is dependent on the whole range of factors, including farming system, nutrition, genetic factors, overall health status of the herd *(Došen et al., 2007;Prodanov-Radulović et al., 2014)*. However, infectious agents may be often introduced into the swine herd by purchasing latently infected animals from another farm with different health status and cause an outbreak of respiratory disease *(Gottschalk and Taylor, 2006). Actinobacillus pleuropneumoniae* (App) is the etiologic agent of porcine pleuropneumonia and one among the most important bacterial pulmonary pathogens in pigs. This is the most common pathogenic bacteria in the pig industry *(Gómez-Laguna et al., 2014).* There is a total of 15 App serotypes discovered and all are capable of causing disease, although there is evidence that some are more virulent than others. These variations may be attributed to the production of different combinations of the Apx toxins *(Gottschalk and Taylor, 2006).* The main route of spread of infection is by direct contact with infected pigs or by aerosols within short distances *(Bosse et al., 2002).* Transmission between different herds occurs through the introduction of carrier animals to naïve populations *(Gottschalk, 2012).* Acute outbreaks of the disease are characterized by a hemorrhagic necrotizing pneumonia and fibrinous pleuritic with high morbidity and mortality. In chronically infected herds, the bacterium causes a decreased rate of weight gain, inefficient feed conversion and increased time to market *(Chiers et al., 2002)*. The inspection at slaughter might be a useful tool for monitoring the health status of animals and data source for further epidemiological studies *(Došen et al., 2014a; Prodanov-Radulović et al., 2015).* This study was aimed at establishing diagnosis of respiratory syndrome on a pig farm characterized by clinical respiratory disease and high mortality rate of fatteners and implementing of appropriate control measures.

Materials and Methods

This study was conducted in a conventional farrow-to-finish herd with 850 sows (Yorkshire x Swedish Landrace). The herd had a history of health respiratory problems in fatteners. On the farm, the piglets are weaned at the age of 28 days. At that age the piglets are transferred to a weaning and after that growing unit with pens. Pens were cleaned before a new group entered the empty pens, but nose-to-nose contact between different age groups of pigs housed in adjacent pens was possible. Pigs are slaughtered at a live weight of approximately 110 kg or at the age of 25 weeks. Vaccination against App was not carried out in the examined herd. The applied research methods included clinical and gross pathological examination of diseased and dead pigs (in total 35). In the case of observation of gross

pathology lesions indicative for respiratory disease, the tissue samples (tonsils, lungs, mediastinal lymph nodes) deriving from dead pigs on the farm were sampled for bacteriological examination. At the slaughterhouse, thoracic cavity organs from in total 360 fatteners that have reached the slaughter body mass were examined and observed gross pathology changes indicative for respiratory infections were recorded. Moreover, the tissue samples of some numbers (in total 170) of altered respiratory organs (lungs, mediastinal lymph node, tonsils) were collected to bacterial examination. The bacteriological examination was performed applying standard bacteriological diagnostic methods *(Quin, 2011)*. From 170 fatteners at the slaughter line, the blood samples were taken in order to perform serological investigation on the presence of specific antibodies against *App* using immunoenzyme (ELISA) test (IDEXX APP-ApixIV) according to the manufacturers instruction. The test is based on the presence of recombinant ApxIV antigen, which enables detection of App indenpedently of bacterial serotype.

Results and Discussion

In the examined farm, clinically severe symptoms of respiratory disease in growing pigs and fatteners were detected. In diseased animals depression was noted together with the respiratory distress, fever and growth disorders. Outbreaks of respiratory disease were frequently observed shortly after growing pigs entered the fattening units.

Clinical sigs of porcine pleuropneumonia vary with the age of animals, their state of immunity, the environmental conditions, and the degree of exposure to the infectious agent *(Bosse et al., 2002)*. In the peracute form, one or more pigs in the same or different pens suddenly become sick with fever, apathy and anorexia. In the terminal phase, there is a severe dyspnea and shortly before death, there is usually foamy, blood-tinged discharge through the mouth and nostrils. The chronic form develops after the disappearance of acute signs. Affected animals can be identified by their intolerance of exercise *(Gottschalk, 2012)*. In chronically infected herds there are many subclinically diseased animals *(Chiers et al., 2002)*. Factors such as crowding, adverse climatic condition and feed quality encourage the development and spread of the App *(Prodanov-Radulović et al., 2014)*.

At post mortem examination severe lung lesions were present in large number of died animals and extensive fibrinohaemorrhagic lung lesions were observed. In chronic infections, the lesions were necrotizing and associated with fibrinous pleurisy. An adhesive pleurisy was observed between the pleura covering these lesions and parietal pleura. By examination of respiratory organs of 360 fatteners at the slaughter line, no visible changes in lung tissue were observed only in 19.44% of examined pigs. In others, examination of respiratory organs revealed

moderate to severe pathological changes indicative for App infection. In most of examined pigs, the changes in the pleura were established, manifested as local pleuritis (32.77%) and chronic diffuse pleuritis (21.11%). Also the pathological signs indicative for App infection *(Pleuropneumonia haemorrhagica necroticans* 7.77%) were discovered. In some number of fatteners, the changes in the heart muscle (*Pericarditis villosa* 8.33%) and signs of purulent infection of lungs (*Pneumonia apostematosa disseminatae* 10.5%) were observed.

The characteristic gross pathological App lesion consists of a mostly bilateral necrotic haemorrhagic pneumonia *(Gómez-Laguna et al., 2014).* The pneumonic areas appear dark red-purple and firm with little or no fibrinous pleurisy. On cut surface there is diffuse hemorrhage and areas of necrosis. In acute cases, layers of fibrin are obvious on the pleural surface and infrequently on the epicardium and pericardium. In chronic cases, fibrosis of the previously fibrinous pleuritic results in firm adhesions between visceral and parental pleura *(Gottschalk, 2012).* It has been demonstrated that a high prevalence of fibrous pleuritic in slaughter is very suggestive of previous App pleuropneumonia *(Gottschalk and Taylor, 2006).*

The serum samples collected at slaughter line were analyzed in the laboratory for the presence of antibodies to App. Serological examination in total 170 blood samples revealed presence of specific antibodies against App in 50% of examined sera samples.

Serology is the most effective tool used to detect subclinical App infections and most cost-effective method for App surveillance *(Gottschalk and Taylor, 2006).* However, serological testing occasionally generates ambiguous results, and bacterial isolation should be carried out. In fact, tonsil colonization without induction of antibody response has been demonstrated *(Gottschalk, 2012).* This confirms that serological assays are of limited value to detect subclinically infected animals *(Sjölund et al., 2011).*

Bacteriological examination in total 170 tissue samples obtained from pigs at slaughter revealed the presence of *Haemophilus parasuis* in 13,53%, *Pasteurella multocida* in 8,24%, *Trueperella pyogenes* in 4,7%, *Staphylococcus aureus* in 0,59%, and *Mannheimia haemolytica* in 0,59% cases. The App was detected in only 2 examined tissue samples (1.18%). In this study, tissue samples were obtained from pigs at the slaughter line which, according to veterinary records did not show any clinical respiratory tract disorders 3-5 weeks before slaughter. The animals had not been treated with antimicrobial agents in the 3 weeks prior to sample collection. Lung samples for culture should come from pathologically changed areas of lungs collected from peracutely or acutely affected untreated animals. In chronic cases, where sequestra are in lungs and fibrous pleuritic is observed, App culture is typically negative *(Gottschalk, 2012).* Although acute

forms of the disease are observed, the most common manifestation following infections with App is pleuritis seen at the post mortem inspection at slaughter *(Gottschalk and Taylor, 2006)*. However, pleuritis does not provide information on the etiological cause of the lesions *(Sjölund et al., 2011)*. Efforts to control respiratory diseases have mainly focused on measures undertaken during the fattening period, and include improvements in environment, antibiotic treatment, vaccination *(Chiers et al., 2002)*. A wide range of vaccines have been developed for this disease (bacterins and subunit toxin-based vaccines). Vaccination of piglets is usually advised but sow and replacement animals can also be vaccinated without adverse effects *(Gottschalk, 2012)*. Antibiotic therapy is most effective in acute outbreaks of the disease, although prevention can be accomplished using continuous or intermittent antimicrobial therapy. However, such treatments can contribute to the induction and spread of antibiotic resistance *(Došen et al., 2014b; Vanni et al., 2012)*. Results obtained so far indicate that bacteria from carrier animals cannot be eliminated under antibiotic treatment. When herds are free of App, strict biosecurity should be practiced to prevent infection *(Gottschalk, 2012)*.

Conclusion

Results of present study confirm that the control at the slaughter line can be a useful tool for the detection of pigs infected with App. Changes in the respiratory organs were evident at high rate in clinically healthy fatteners, resulting in pronounced inconsistency in body mass and carcass quality. The obtained results strongly suggest the necessity of implementation of a vaccination program, taking into consideration the isolated causative bacterial agents.

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Kontrola i dijagnostika respiratornog oboljenja svinja uzrokovanog *Actinobacillus Pleuropneumoniae*

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Rezime

Actinobacillus pleuropneumoniae (App) je etiološki uzročnik pleuropneumonije i jedan od najznačajnijih bakterijskih patogena respiratornog trakta svinja. Cilj istraživanja je bio dijagnostika respiratornog oboljenja utvrđenog na farmi svinja i primena mera kontrole. Metode ispitivanja su obuhvatale klinički i patomorfološki prelged obolelih i uginulih svinja, i kontrolu respiratornih organa na liniji klanja. Na liniji klanja je vršeno uzorkovanje organa (tonzile, deo pluća i limfni čvor) u cilju bakteriološkog ispitivanja i uzorkovanje krvi za ispitivanje na prisustvo antitela protiv App. Pregledom respiratornih organa od ukupno 360 tovljenika, u 19,44% nisu utvrđene vidljive promene na respiratornom traktu, dok su kod preostalih utvđene patološke promene koje ukazuju na infekciju sa App (pleuritis, *Pleuropneumonia haemorrhagica necroticans*). Serološkim ispitivanjem (ELISA test) od 170 uzoraka seruma, u 50% je utrvđeno prisustvo antitela na App. Bakteriološkim ispitivanjem ustanovljeni su sledeći mikroorganizmi: *Haemophilus parasuis*, *Pasteurella multocida*, *Trueperella pyogenes*, *Staphylococcus aureus*, *Mannheimia haemolytica*. Prisustvo App je potvrđeno samo u 2 ispitana uzorka. Rezultati istraživanja potvrđuju da kontrola svinja na liniji klanja i serološka ispitivanja mogu biti korisni u dijagnostici svinja inficiranih App.

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DETECTION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN DUST SAMPLES IN PIG FARMS IN SERBIA

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Original scientific paper

Abstract: *Staphylococcus aureus* (*S.aureus*) belongs to the ubiquitous and very adaptable microorganisms. The methicillin-resistant variant of *S.aureus* (MRSA) is resistant to β-lactam antibiotics, including penicillins, cephalosporins, carbapenems and their derivates. Isolation of MRSA from animals is reported worldwide and involves many animal species such as cattle, swine ,dogs, cats, horses and poultry. Isolation of MRSA from animals was first reported in 1972 following its detection in milk from dairy cow. In 2005, the presence of MRSA in pigs and the transfer to humans was reported for the first time. In recent years, livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA), has emerged in diffrent animal species. LA-MRSA can be detected in dust of facilities and exhaust air from pig facilities as well as in surrounding soil.In our examination we tested 50 dust samples and found 9 strains of *S.aureus*.According to phenotypic and genotypic tests a total of four MRSA strains were identified in this study. All nine strains *S.aureus* has growth as colonies with the double haemolysis zone on blood agar and only four MRSA strains has growth as green colonies on chromogenic media -chromID MRSA. In this study, three of five investigated farms were MRSA positive. Pig farms with large agglomeration of animals within small spaces represent ecological niches in which bacteria are permanently exposed to different antibiotics. This instigates the occurrence of multiresistant strains of bacteria.The obtained results point that it is indispensable to undertake the necessary measures to prevent MRSA strains spread among pigs, as this will reduce the health risk for veterinarians, farm workers and their families.

Key words: MRSA-methicillin-resistant Staphylococcus aureus, dust, pig.

Introduction

Staphylococcus aureus (*S.aureus*) belongs to the ubiquitous and very adaptable microorganisms.*S.aureus* may be found on skin and mucous membranes of humans and animals, as well as in air,soil and water.It can cause a wide range of conditions in humans and animals, from mild skin infections to fatal invasive disease *(Leonard and Markey,2008).*The methicillin-resistant variant of *S.aureus* (MRSA) is resistant to β-lactam antibiotics, including penicillins, cephalosporins, carbapenems and their derivates *(Doyle et al., 2012).* Resistance to β -lactam in MRSA is conferred by the acquisition of a mobile genetic element, the staphylococcal cassette chromosome (SCCmec) carrying the mecA gene which encodes an altered PBP – PBP2a/PBP2 which has reduced affinity for β -lactam antibiotics *(Paterson et al.,2014).*

Isolation of MRSA from animals was first reported in 1972 following its detection in milk from dairy cow *(Devriese et al., 1972).* In 2005, the presence of MRSA in pigs and the transfer to humans was reported for the first time (*Voss et al., 2005*).

In recent years, livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA), has emerged in different animal species and humans who were in direct contact with animals and also humans without contact *(Kadlec et al., 2013; From et al.,2014).*

Research has focused on livestock-associated methicillin-resistant Staphylococcus aureus specially of the clonal complex 398 and their dissemination between humans and animals *(Kadlec et al.,2013; Alt et al.,2011).* LA-MRSA can be detected in exhaust air from pig barns and in soil samples at least 300 m from the barn *(Skov, 2013)* suggesting important role of air exposure and transmission for MRSA *(Bos et al., 2013).*

Recent studies have shown that MRSA ST398, also referred to as livestock associated MRSA, occurs with high prevalence in pig holdings in several European countries.(*Weese and Van Duijkeren.,2010*).In the Netherlands, living in livestock-dense areas was recently identified as a risk factor for MRSA CC398 carriage, independently of whether the carrier had direct livestock contact *(Feingold et al.,2012).* Also,persons who are frequently in contact with pigs and other production animals through their profession are more frequently colonised with MRSA than the general population *(Bfr,2013).*

MRSA is present in the nose and on the skin by infected or colonized people and animals, and into the environment indicating that airborne transmission is a possible route for infection.

Airborne emission of MRSA from livestock farms has been reported in Germany *(Schulz et al., 2012).* The problem of MRSA colonization in food

producing animals and the links with human infection have an impact both on food production and on health of people that work with animals with possible risks of disease for the general population *(Visciano et al., 2014)*.

The major problem lies with the outbreak of a MRSA infection, in which case the choice of antibiotic for therapy is narrowed down to one or several antibiotics.

In the farms MRSA spread by direct contact between animals. Dust and contaminated surfaces may be reservoirs for MRSA. MRSA have a selective advantage and may spread and persist if antibiotics is used *(Broens et al., 2011)*. It has been established that MRSA can be found in dust and in the air of pig barns and may be emitted via the exhaust air of animal houses into the environment *(Friese et al., 2012)*.

Previous studies in our country, were detected MRSA in nasal swabs *(Velebit et al., 2010)* and clinical samples originating from pigs *(Zutic et al.,2012)*.The aim of this work was to evaluate the deposition of MRSA containing dust inside the pig farms.

Materials and method

A total of 50 dust samples were taken from five pig farms in Serbia. On each farm, 10 dust samples were taken from the facilities with animals. Each swab was used to sample approximately 400 cm2 surface area with dust and stored in sterile test tubes. On the same day samples were inoculated for pre-enrichment in Muller Hinton broth (Himedia) supplemented with 6.5% NaCl and incubated 24 h at 37^{o}C in aerobic condition. After 24 h, one loop-full was inoculated on the surface of sheep blood agar and chromogenic culture media (chromID MRSA, bioMerieux).All plates were incubated aerobically at 37^{0}C and examined for growth at 24 h.

Colonies suspicious to *S. aureus* on the blood agar and green colonies on chromogenic media were transfered to individual plate to make pure culture. *S.aureus* were identified by using colony morphology, Gram staining, catalase, coagulase and hemolytic pattern on blood agar. The identification was confirmed using BBL Crystal, G/P, ID kit (Becton Dickinson).

Antimicrobial susceptibility testing was performed by disk diffusion method with cefoxitin discs 30 µg (Rosco, Denmark) in accordance to the Clinical and Laboratory Standard Institute recommendations (*CLSI 2008*).*Staphylococcus aureus* ATCC 25923 was used as the control strain. All *S. aureus* isolates were tested for presence of *mec*A gene by PCR *(Bignardi et al., 1996)*.

Results and discussion

In this examination we tested 50 dust samples and found 9 strains of *S.aureus*.According to phenotypic and genotypic tests a total of 4 MRSA strains were identified in this study. Positive and negative findings of this research are summarized in Table1. All nine strains *S.aureus* has growth as colonies with the double haemolysis zone on blood agar (Figure1) and only four MRSA strains has growth as green colonies on chromogenic media -chromID MRSA (Figure 2).

Table 1. Number of positive and total dust samples on farms

Farm No	Positive samples on S.aureus	Positive samples on MRSA	Total samples
1.	3	1	10
2.	0	0	10
3.	3	1	10
4.	1	0	10
5.	2	2	10
Total	9	4	50

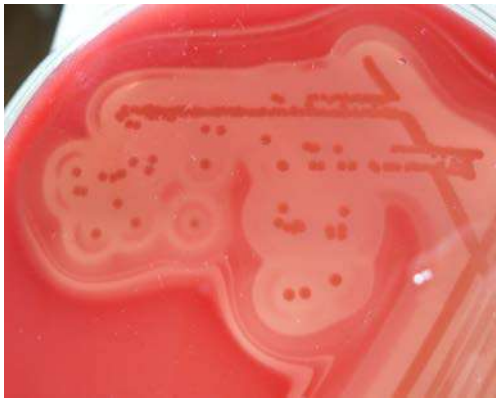

Figure 1. S. aureus-colonies on blood agar agar

Figure 2. MRSA- colonies on chromID

In this study, three of five investigated farms were MRSA positive. In the farm number five it has already been determined MRSA *(Žutić et al., 2012)*. Our results are similar with the results in Croatia where the four of the six examined farms were positive *(Habrun et al., 2012).* They tested 32 dust samples and found eight MRSA isolates.During the research in Norway, dust sample was positive

from the building where the pigs are also MRSA positive. Dust samples, from the building MRSA negative animals, were negative too *(Sunde et al., 2011)*. These findings suggest that a pig carriers have a significant role for MRSA transmission in the environment. The research in Netherlands showed that 56% farms (sample originated from pigs or stable dust) were positive *(Van Den Broek et al.,2008)*. Other research showed the similar results. Very high percentage of positive fattening pigs farms (52%) was found in Germany *(Alt et al., 2011)*.

Methicillin-resistant *S. aureus* can colonize livestock, livestock workers and person who live in areas of high livestock density in the US and Europe *(Carrel et al., 2013)*. Farmers and veterinarians in contact with livestock are the main risk groups for colonization with LA-MRSA.Also, living in livestock-dense areas was recently identified as a risk factor for MRSA carriage in The Netherlands, independently of whether the carrier had direct livestock contact *(Feingold et al.,2012)*.The emerging problem of MRSA colonization in food producing animals and the links with human infection have an impact both on food production and on health of people that work with animals with possible risks of disease for the general population *(Fitzgerald, 2012;Visciano et al.,2014)*.

Strains with a wider ecological resistance have a enabling them to thrive in various interconnected ecological niches. The fact that resistant microorganisms can survive in a wide range of potential niches and adapt to alternative hosts and this could amplify their capability to acquire new determinants of resistance *(Da Costa et al., 2013)*.

Pig farms with large agglomeration of animals within small spaces represent ecological niches in which bacteria are permanently exposed to different antibiotics. This instigates the occurrence of multiresistant strains of bacteria that, via insects, mice, rats, wastewater, and even farm workers, spread further to other plant and animal species, as well as to the surrounding soil. At the same time, there is a strong possibility that the gene of resistance can be transferred to commensal microorganisms and from them to a huge bacterial population.

The use of antibiotics on pig farms is an exceptionally complex process that includes therapy and metaphylaxis.Antibiotics are usually administered to animals in a group in water or feed that makes difficult an appropriate dosage for an individual animal. The method actually creates an opportunity for a subdosage of antibiotics and potential development of antimicrobial drug resistance.What happens is that during the therapy the antibiotic inevitably kills susceptible non-pathogenic bacteria too, that are a constituent part of physiological microbiota of the organism. This is a selective pressure where useful bacteria are killed, and only resistant species survive.

The obtained results point that it is indispensable to undertake the necessary measures to prevent MRSA strains spread among pigs, as this will

reduce the health risk for veterinarians, farm workers and their families. In addition, it will provide protection for slaughterhouse workers end consumers too.

Conclusion

The increase in the incidence of methicillin - resistant *S.aureus* strains poses a very serious problem in human and veterinary medicines. Thence there is the necessity for an early and reliable detection of this type of resistance. The colonization of pigs with MRSA strains, as well as contamination of their environment, present a serious threat to the spread of the strains to other animals and also a possible source of infection for humans, primarily those working on the farm or living in the immediate vicinity. This is why it is obligatory to continually monitor the issue in order to be able to provide the necessary recommendations and programs on the basis of the relevant data with an aim to prevent the spread of resistance and lower its current levels.

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Detekcija meticilin-rezistentnog Staphylococcus aureus u uzorcima prašine na farmama svinja u Srbiji

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Rezime

Staphylococcus aureus (*S.aureus*) pripada grupi ubikvitarnih i veoma adaptabilnih mikroorganizama. Meticilin-rezistentni *S.aureus* (MRSA) poseban je soj ove vrste koji je razvio rezistenciju na više različitih antibiotika. Iako se meticilin više ne koristi u terapiji stafilokoknih infekcija, ostao je akronim MRSA, a odnosi se na *S.aureus* koji je rezistentan na sve β-laktamske antibiotike uključujući cefalosporine i karbapeneme.

MRSA je izolovan iz domaćih životinja kao i ljudi koji su bili u kontaktu sa životinjama kao rezervoarima ovog patogena pa je stoga i nazvan LA-MRSA (Livestock-associated MRSA). Prvi izveštaj o prisustvu MRSA sojeva kod

životinja datira još iz 1972.godine kada je meticilin rezistentni *S.aureus* izolovan iz mleka krava sa mastitisom. Infekcije svinja sojevima MRSA prvi put su opisane u Holandiji 2005. godine, pri čemu su svinje bile izvor infekcije za ljude.Početkom ovog veka porastao je broj izveštaja o prisustvu stafilokoka rezistentnih na antibiotike kod bolesnih, ali i kod zdravih domaćih životinja. Istovremeno, otkriveno je njihovo prisustvo u objektima u kojim životinje borave kao i u okolini tih objekata.Imajući u vidu ovu činjenicu, cilj ovog rada bio je da utvrdimo eventualno prisustvo MRSA sojeva u prašini u objektima za uzgoj svinja

Uzorke prašine prikupili smo sa 5 svinjarskih farmi u Srbiji iz objekata u kojima su smeštene svinje različitih kategorija (odgoj, tov). Na svakoj farmi uzeli smo po 10 uzoraka.Iz ukupno 50 uzoraka prašine ,izolovali smo 9 sojeva *S.aureus* sa 4 farme. Među njima, a na osnovu fenotipskih i genotipskih karakteristika, identifikovali smo 4 MRSA soja koja su poticala sa 3 farme. Na jednoj od tih farmi smo u prethodnom periodu u kliničkim uzorcima ustanovili prisustvo MRSA sojeva.Sva 4 soja rasla su u vidu zelenih kolonija na hromogenoj chromID MRSA podlozi. Ostalih 5 sojeva stafilokoka nisu rasli na ovoj podlozi što ukazuje na njeno selektivno svojstvo. U uzorcima prašine sa dve farme nije ustanovljeno prisustvo MRSA sojeva. Farme svinja sa velikom koncentracijom životinja na malom prostoru predstavljaju ekološke niše u kojima su bakterije vrlo često izložene uticaju različitih vrsta antibiotika.To favorizuje pojavu multirezistentnih sojeva bakterija koje se, šire dalje na biljne i životinjske vrste, kao i okolno zemljište.

Kolonizacija svinja sojevima MRSA kao i kontaminacija njihove okoline predstavlja potencijalnu opasnost širenja sojeva na ostale jedinke u populaciji ali i mogući izvor infekcije za ljude, primarno za one koji rade na farmi kao i članove njihovih porodica.

I pored podataka o niskoj prevalenciji MRSA sojeva kod svinja, smatramo da su neophodna obuhvatnija istraživanja u cilju kontrole rezistencije MRSA sojeva kao i rezistencije ostalih značajnih bakterijskih patogena prisutnih u populaciji svinja u našoj zemlji.

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COMPARISON OF ESSENTIAL METALS CONTENT IN IMPORTED AND DOMESTIC PORK IN SERBIA

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Original scientific paper

Abstract: Pork is valuable source of proteins, essential vitamins and minerals for human diet. Pork consumption in Serbia has a high share of total meat consumption. Most Serbian pig production corresponds to intensive indoor industrial farming, but in the past few years an increase of breeding pigs in extensive systems has been recorded. Besides national production, significant amounts of all meat types are imported, mainly from EU countries. The aim of this work was to determine essential elements' content (Cu, Mg, Zn and Fe), as well as to compare the content of analysed elements in imported pig meat from Spain, Germany and France to the meat of pigs grown in Serbia in both breeding systems (intensive and extensive).The content of chemical elements was measured in 64 samples of pork shoulder. The analysis of the following isotopes was carried-out: copper (^{63}Cu), magnesium (^{24}Mg), zinc (^{66}Zn) and iron (^{57}Fe). Analytical technique was inductively coupled plasma mass spectrometry (ICP-MS). Obtained results of this study showed that there were no statistically significant differences in Cu content, as well as in Mg content between all analysed samples. Shoulder samples of pig grown in extensive system in Serbia had significantly higher content, both of Zn and Fe, than shoulder samples of imported pigs from Germany, Spain and France, as well as samples of pigs grown in intensive system in Serbia. Therefore, it can be concluded that different types of breeding can influence the content of essential elements status, regardless from origin of the animals.

Key words: Pork, Cu, Mg, Zn, Fe, ICP-MS

Introduction

Pork is valuable and convenient source of proteins, vitamins and essential minerals for human diet. Chemical composition of meat depends on age, physiological status, type and feeding regimes, as well as on breeding conditions. In order to promote animal growth and prevent diseases, many chemical elements are added to animal feed excessively *(Zahran and Hendy, 2015)*. Minerals represent nearly 1% of meat, including micro- and macro- elements *(Šutiak et al., 2000).*

Zinc is an essential element in human diet. Zinc deficiency in diet causes problems such as depression *(Mc Loughlin and Hodge, 1990; Maes et al., 1997)* and accumulation of cadmium, toxic element, in some human tissues and organs *(Brzoska and Moniuszko-Jakoniuk, 2001).*On the other side, high content of zinc in food can be harmful to human health *(Agency for toxic substances and Disease registry, 2005)*. Copper is also essential for a number of biochemical processes, however, high content in food could cause health problems *(Agency for toxic substances & Disease registry, 2005)*. Iron is present in food as heme and non-heme iron. Heme iron is found in meat and is the form of iron that is most readily absorbed from the stomach and taken up into the body after the meal. Magnesium has a vital function in numerous metabolic reactions and represents an activator for many enzymatic reactions. Dietary magnesium supplementation positively affects behavior and decreases stress. Its deficiency causes hypocalcaemia, cardiac and nervous manifestations *(Ryan, 1991; Swaminanthan, 2003).*

Pork consumption has a high share of total meat consumption in Serbia. According to the FAO data *(Food and Agriculture Organization of United Nations, 2008)* the average meat consumption in Serbia is as follows: pork – 25 kg, beef – 8 kg, poultry – 18 kg, and is similar compared to the EU countries (pork – 42,6 kg, beef – 17,4 kg, poultry – 16,3 kg). Most Serbian pigs' production corresponds to intensive indoor industrial farming but in the past few years, there has been an increase in breeding pigs in extensive systems. Beside national production, meat on Serbian market is imported from EU.

The aim of this study was to determine essential elements' content (Cu, Mg, Zn and Fe), as well as to compare the content of analysed elements in imported pig meat from Spain, Germany and France with the meat of pigs grown in Serbia in both breeding systems (intensive and extensive).

Materials and Methods

The content of Cu, Mg, Zn and Fe were measured in 64 samples of pork shoulder: 31 imported shoulders (Spain - 10, Germany - 12, France - 9) as well as 17 samples originating from intensive pigs farm from Serbia, were collected from the Serbian market; 16 samples were taken from pigs grown in extensive system (Bojčinska šuma, Serbia). Samples were collected from September 2014 to April 2015. All meat samples were packed individually in plastic bags and stored at -18 ºC until analysed.

Frozen samples were thawed before analysis and subsequently homogenized in commercial blender (Bosch, MMR501). Aliquots of approximately 0.3 g were transferred into PTFE vessels and treated with 5 mL nitric acid (67% Trace Metal Grade, Fisher Scientific, Bishop, UK) and 1.5 mL hydrogen peroxide (30% analytical grade, sigma-Aldrich, St. Louis, MA, USA). Microwave assisted digestion was performed using Microwave Digestion System (Start D, Milestone, Sorisole, Italy). The digested sample solutions were quantitatively transferred into volumetric flasks and diluted to 100 mL with deionized water (ELGA, Buckinghamshire, UK).

The analysis was performed by inductively-coupled plasma mass spectrometry (ICP-MS). Measurements were performed using the instrument "iCap Q" (Thermo Scientific, Bremen, Germany), equipped with collision cell and operating in kinetic energy discrimination (KED) mode. The following isotopes were measured: copper (^{63}Cu), magnesium (^{24}Mg), zinc (^{66}Zn) and iron (^{57}Fe).Torch position, ion optics and detector settings were re-adjusted daily using tuning solution (Thermo Scientific Tune B), in order to optimize mechanical and electrical parameters and minimize possible interferences. A five-point calibration curve (including zero) was constructed for each isotope: ^{63}Cu, ^{24}Mg, ^{66}Zn and ^{57}Fe, in the concentration range of 0.1–2.0 mg/L. All solutions were prepared in 2% nitric acid. Multielement internal standard (^{6}Li, ^{45}Sc-10 ng/mL; ^{71}Ga, ^{89}Y, ^{209}Bi-2 ng/mL) was introduced online by other line of the peristaltic pump. Measured concentrations were corrected for response factors of internal standards. The quality of the analytical process was confirmed by the analysis of the standard reference materials SRM 1577c and SRM 2384 (Gaithersburg, MD, USA) and were within the range of the certified values.

Statistical analysis was performed using the Minitab 16.0 software. One-way (unstacked) ANOVA analysis of variance and Tukey's test was used in order to compare the differences in element content between shoulder samples.

Results and Discussion

The content of Cu, Mg, Zn and Fe in all analysed samples is shown in Table 1. Content was expressed as mean ± standard deviation, minimum and maximum values.

Table 1. Content of Cu, Mg, Zn, and Fe (mg/kg) in pork shoulder (n=64)

		Cu	Mg	Zn	Fe
Country of origin	n	Mean ± SD (ranges)			
Serbia (extensive)	16	1.6 ± 0.2 (1.3-2.3)	209.7 ±14.8 (176.5-233.7)	44.8± 7.4 [a] (34.3-60.1)	19.6 ± 5.0[a] (12.3-27.8)
Serbia (intensive)	17	0.8 ± 0.3 (0.2-1.3)	208.5 ±29.2 (102.0-380.7.7)	27.5± 12.2[b] (8.51-50.6)	8.8 ± 4.1[b] (2.8-20.3)
Germany	12	1.2 ± 0.5 (0.4-2.1)	218.9 ± 34.6 (158.5-272.0)	29.0± 13.0[b] (10.8-47.4)	9.3± 3.5[b] (3.9-14.8)
Spain	10	0.8 ± 0.2 (0.6-1.2)	216.1 ± 25.7 (188.8-266.2)	25.3± 7.7[b] (15.7-42.1)	6.4± 2.1[b] (2.6-10.1)
France	9	0.8 ± 0.2 (0.5-1.2)	217.8 ± 25.1 (196.4-269.0)	24.3± 11.0[b] (12.7-42.8)	7.7± 3.6[b] (4.1-15.2)

[a,b]Values in the same column followed by the different letters are significantly different ($p<0.05$)

The mean content of Cu in analysed samples ranged from 0.8 mg/kg to 1.6 mg/kg. The obtained values of Mg content were similar in all shoulder samples and were averagely 214 mg/kg. Data for Cu and Mg content from this study, both in meat samples originating from Serbia and EU countries (Spain, Germany and France), were within the range described in the literature, except for Cu in Lopez-Alonso's study (Table 2). Statistical analysis showed that there were no significant differences ($p>0.05$) in Cu content, as well as in Mg content, between all groups of shoulder samples.

Table 2. Overview of the Cu, Mg, Zn, and Fe content in pork from different countries

Country	Cu	Mg	Zn	Fe	Reference
	[mg/kg]				
Denmark	1.0	210	36	8.30	*Danish Food Composition Databank*
North-West Spain	6.85	-	42.5	26.5	*Lopez-Alonso et al., 2007*
Italy	0.5	-	15.4	4.2	*Lombardi-Boccia et al., 2005*
Poland	1.1	-	26	13	*Falandsyz, 1993*
USA	-	260	19.5	8.9	*United states Department of Agriculture (USDA)*

The lowest content of Zn was measured in pork from France (24.3 mg/kg), while the highest content of Zn was recorded in meat of Serbian pig grown in

extensive system (44.8mg/kg) and it was the only significantly different result ($p<0.05$). Content of Zn obtained in pork extensively grown in Serbia are higher than values from USA, Italy and Poland (Table 2). However, *Lopez-Alonso et al. (2007)* study and *Danish Food Composition Databank* have reported similar results for Zn content in meat (Table 1, 2).

Similar finding, as in Zn case, was obtained for Fe content in meat of extensively breeded pigs. Namely, the content of Fe in meat of Serbian extensive pigs was only significantly different ($p<0.05$) in comparison with Fe content in all groups of shoulder samples. Comparing the results obtained in this study (Table 1) with the literature data for Fe content in meat from Poland, Italy, Denmark and USA (Table 2), it could be concluded that the extensively breeded pigs in Serbia have the highest content of Fe in shoulder meat.

Conclusion

On the base of the results of this study, it can be concluded that there were no statistically significant differences in Cu content, and Mg content between all analysed samples. In the case of Zn and Fe, obtained results showed the following: shoulder samples of pigs grown in extensive systems in Serbia had significantly higher content, both of Zn and Fe, compared to shoulder samples of pigs imported from Germany, Spain and France, as well as samples of pigs grown in intensive systems in Serbia. Therefore, it can be concluded that different types of breeding can influence the content of essential elements status, regardless from origin of the animals.

Poređenje sadržaja esencijalnih elemenata u mesu domaćih i uvezenih svinja u Srbiji

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Rezime

Svinjsko meso je bogat i veoma pogodan izvor proteina, esencijalnih vitamina i minerala u ljudskoj ishrani. Mineralne komponente čine skoro 1% mesa uključujući miko- i makro- elemenete. U odnosu na ostale vrste mesa, svinjsko meso ima veliku potrošnju u Srbiji. Svinje se u Srbiji najviše uzgajaju na farmama pod zatvorenim i kontrolisanim uslovima (intenzivno gajenje). Međutim, u

poslednje vremese sve više uzgajaju slobodno u ruralnim predelima (ekstenzivno gajenje). Pored domaće proizvodnje, meso na srpskom tržištu je i uvezeno iz zemalja Evropske unije kao što su Španija, Nemačka i Francuska. Cilj ovog rada je bio da se utvrde sadržaji esencijalnih elemenata (Cu, Mg, Zn iFe) u plećkama uvezenih i domaćih svinja gajenih,ekstenzvno i intenzivno, u Srbiji. Takođe, statističkom obradom dobijenih podataka, poređen je sadržaj analiziranih esencijalnih elemenata.Ukupno je analizirano 64 uzorka. Uzorci plećki su uzeti od ekstenzivno gajenih svinja u Bojčinskoj šumi(Srbija), (n=16), intenzivno gajenih svinja u Srbiji (n=17) i uvezenih svinja iz Nemačke(n=12), Španije(n=10) i Francuske (n=9).Svi uzorci uzeti su i analizirani u periodu od Septembra 2014 do Aprila 2015. Analiza sadržaja esencijalnih elemenata urađena je primenom *i*nduktivno-kuplovane plazme sa masenom spektrometrijom(ICP-MS), merenjem izotopa bakra (^{63}Cu), magnezijuma (^{24}Mg), cinka (^{66}Zn) i gvožđa (^{57}Fe).Dobijeni rezultati su pokazali da ne postoji statistički značajna razlika ($p<0,05$) u sadržaju Cu, kao ni u sadržaju Mg između svih analiziranih uzoraka plećki. U slučaju sadržaja Zn i Fe utvrđena je statistički značajna razlika($p>0,05$): sadržaji oba elementa su značano veći u plećkama ekstenzivno gajenih svinja u Srbiji u odnosu naplećke intenzivno gajenih svinja u Srbiji, kao i u uvezenih iz zemalja EU. Dakle, može se zaključiti da sadržaj esencijalnih elemenata u svinjskom mesu zavisi od načina uzgoja ali ne i od porekla svinja.

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MORPHOLOGICAL CHARACTERIZATION OF THE HONEY BEE (*Apis mellifera* L.) FROM DIFFERENT SITES OF SERBIA

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Abstract: Serbia has a heterogeneous relief, climatic conditions and honey bee pastures in which honey bees are reared. Due to the adaptability of bees to specific habitat conditions, it is assumed that different geographic ecotypes of bees were created in Serbia. In addition, during the previous decades the natural populations of Carniolan honey bee have been subjected to influence of man through migratory beekeeping and trade in honey bee queens. Therefore, the main aim of this investigation was to analyze part of morphological characters in order to help distinguish and preserve indigenous honey bee varieties. Samples were collected from six different locations covering the territory of Republic of Serbia and compared with honey bee sample from Slovenia. Fifteen worker bees of each sample were dissected and twenty six morphometric characters were measured with stereo microscope Leica XTL-3400D, and software package IL 1009 in accordance with the standard method. The results showed significant differences between the groups of bees. This separation was the most contributed by the characters: angles on the front and hind wing, width of the forewing and length of the third tergit. The morphometrical control turned out to be useful method to a clearer separation of groups of bees inside domestic population.

Key words: honey bee, morphometry, ecotype, Serbia

Introduction

Serbia has excellent prerequisites for the development of beekeeping, distinguished by heterogeneous relief and climatic conditions and by the existence of various honey bee pasture.

Apis mellifera L. is an indigenous species in Europe, Africa, and Asia (including Saudi Arabia, Iran, and the Ural Mountains in Russia). Today, this species is widely disseminated in the world due to multiple migrations and introductions. *Apis mellifera* has about 29 subspecies in different regions of the world *(Munoz et al., 2009)*. On the basis of morphological traits, these subspecies are classified into four main branches *(Ruttner, 1988; Ruttner, 1992)*. The Central and Southeast European "C-subspecies" consisted of honeybee subspecies: *A. m. ligustica, A. m. cecropia, A. m. macedonica* and *A. m. carnica (De La Rua et al., 2009)*.

Based on morphometrical analyses, *A. m. carnica* Poll. covers large territory from southern Austria, Slovenia, Croatia, Bosnia and Herzegovina, Serbia, Montenegro, parts of Hungary, Romania and Bulgaria (Ruttner, 1988). The introgression line with the neighboring populations of *A. m. macedonica* is thought to lie within the territory of the Republic of Macedonia *(Uzunov et al., 2009)*. However, *Stevanović et. al (2010),* in studies of mtDNA (COI-CPII region and COI gene segment) in honeybees (*A.m. carnica* and *A.m. macedonica*) from Serbia, Bosnia and Herzegovina and Republic of Macedonia, reported that honey bees from east part of Serbia, part of south Serbia, south west part of Serbia and Republic of Macedonia belong to the *A. m. macedonica. Pihler et al. (2014*) stated that there are no two types of bees or two separate populations bees from north part of country (Vojvodina).

Morphometry represents very powerful tool for the identification of bee species and subspecies *(Francoy et al., 2006)*. The first attempts to classify bee subspecies were based on color and size *(Ruttner et al., 1978)*. For more complete morphological analysis, *DuPraw (1965)* introduced the measurement of 11 angles on the front wing and noted that these angles as well as other morphometric characters contribute to the classification of samples of different subspecies of honeybees *(Stevanović, 2002)*. These characters are somewhat influenced by environmental conditions and, thus, represent good indicators of genetic relationships between populations *(Meixner et. al., 2007)*.

A number of varieties of carnica have been described *(Ruttner, 1988)*. The first written record of the varieties of honey bees in Serbia can be found in the work of *Živanović (1893).* Later, *Grozdanić (1926)* suggested the separation of the bees from Banat into subspecies *A. m. banatica* based on morphological comparisons of honey bee samples from Banat with *A. m. carnica, A. m. ligustica, A. m. cypria* and *A. m. syriaca*. Numerous authors have continued studies of the morphometric variation within honeybee population in Serbia *(Vlatković, 1957; Konstantinović, 1965; Krunić, 1967; Plužnikov, 1995; Nedić et al., 2007; Mladenović and Simeonova, 2010; Nedić et al. 2011)*.

During the previous decades the natural populations of Carniolan honeybee in Serbia have been subjected to natural crossbreeding with the neighboring

subspecies *A. m. macedonica*. Also, moving and migrating beekeeping, queen replacement with those of unknown origin and the influence of human activity through the uncontrolled importation of foreign queens and crossbreeding with indigenous populations also contribute to the hybridization. Today there is also the view that only two populations of honey bees can be considered in Serbia: gray bees (*Apis mellifera carnica* Poll.) and yellow bees (*Apis mellifera banatica*) (Mladenović and Simeonova, 2010). In order to preserve biodiversity in the Republic of Serbia, cultivation and trade in breeding material from other races except the bees *Apis mellifera carnica* is not allowed *(Livestock law, Official Gazette of RS 41/09)*. Morphometric methods are relatively simple and inexpensive way to take the first step in selection of the bees under the prescribed standard for the subspecies.

Modern honeybee populations in many parts of the world are mostly hybrids between native and introduced populations *(Sušnik et al., 2004)*. Considering that the beekeepers are mostly interested in the economic traits of bees maintaining of biodiversity can be difficult. It should be noted that the impact of man on the change in the bio-geographic distribution of individual races of bees in Europe in the past was very important. Therefore, in practice interest in more productive bees can overcome interest in maintaining indigenous biological materials.

It is important to identify variation within Serbian honey bee population, in order to help distinguish and preserve indigenous Carniolan honeybee. The aim of this study was to analyze the size of the 26 morphometric traits of honey bee samples from different locations of the territory of Serbia and compare them with characters measured at sample of honeybee from Slovenia.

Materials and Methods

Samples of 15 adult worker bees (*Meixner et al., 2007*) were collected from 6 different apiaries along Serbia: Središte (45^{o}09´02.79´´N, 21^{o}24´14.09´´E), Kljajićevo (45^{o}45´59.42´´N, 19^{o}17´00.23´´E), Bajina Bašta (43^{o}58´17.81´´N, 19^{o}33´31.88´´E), Aleksandrovac (43^{o}27´38.88"N 21^{o}03´13.97"E), Babušnica (43^{o}03´47.85´´N, 22^{o}24´52.21´´E), Kosovska Mitrovica (42^{o}55´02.14´´N, 20^{o}48´34.38´´E) and the seventh sample originated from Slovenia. The distant sample locations in Serbia were chosen to represent different parts of the country with different ecological conditions. Bees were preserved in 95% ethanol, and then dissected, and the right forewing, right hindwing, right hind leg, and third tergite were mounted on glass slides *(Rinderer, 1991)* and measured using Leica XTL-3400D binocular microscope and software package IL 1009 in accordance with the standard method by Ruttner et al. (1978).

The following characters were used for measurements: angle A4, angle B4, angle D7, angle E9, angle L13, angle J10, angle J16, angle N23, angle K19, angle G18, angle O26, angle W1, angle W2, angle W3, cubital vein lenght a (CUB a), cubital vein lenght b (CUB b), forewing length (FWL); forewing width (FWW), hind wing length (HWL), hind wing width (HWW), tergit 3 width (TEW), tergit 3 lenght (TEL), femur length (FEL), tibia length (TIL), basitarsus lenght (BAL) and basitarsus width (BAW).
Univariate (variance) statistical analyses were conducted for 26 morphological traits of worker bee samples from different locations of the territory of Serbia and Slovenia. A descriptive statistical analysis was carried out and comparisons between locations were determined by Duncan´s Studentized Multiple Range Test. All measurements of morphological characteristics for the bees were analysed by multivariate discriminant analyses.

Results and Discussion

The values of descriptive statistics of analyzed morphometric characters are shown in Table 1. The results of the measurements of the sampled worker bees showed wide variations of the means and standard deviations for the examined morphometrical characters. Size of angle A4 varied from 27.64 to 29.85°, with an average of 29.22°. Bees from Western Serbia (III) differed significantly for this character ($P < 0.01$) from groups II, IV and VI of bees from Serbia and a sample of bees from Slovenia (VII). According to *Nedić et al. (2011),* the bees from seven sites across Serbia have an average size of A4 angle of 29.80 ± 1.87. In studies of *Stevanović (2002),* bees from Banat, Timok, Sjenica and Pešter ecotypes had the angle A4 31.3±0.38, 29.8±0.85, 29.2±0.98, respectively. Average values for angles B4, D7, E9 and L13 were 111.59±5.13, 97.90±3.11, 23.82±1.78 and 14.75±1.37, respectively. Determined differences of average values of these angles between the groups of bees from different sites in Serbia and a group of bees from Slovenia were not statistically significant. However, the average value of angles J10, J16, N23, W1 and W2 were statistically very significantly ($P < 0.01$) and contributed to intergroup variability.
Beside certain angles, the discrimination of two groups of bees was very significantly influenced by the other studied morphometric characters on the wings. The data obtained by descriptive statistics (table 1) show that there are variations from 9.014 to 9.319 mm in the length of the front wing. These values correspond with results of *Krivcov (1992)* which state that the length of front wing in Carniolan race ranges from 9.00 to 9:40 mm. Also, a significant difference in the width of the front wing was found between two groups of bees and it ranged from the 3.161 to 3.311 mm, with a mean of 3.237 mm.

Jevtić (2007), exploring the bees from six different sites in Serbia, noted that the minimum width of the front wings was in bees from central Serbia (3.09 mm), and maximum bees from eastern Serbia (3.38 mm). By analyzing the sampled bees of Apis mellifera carnica from Austria, *Kandemir et al. (2004)* stated that an average value of wing width was 2.983±0.017 mm. The results of the factor of length and width of the third tergite significantly contributed to the total variability. By analyzing the results of these characters, it was found that the bees from group II (Banat) were very significantly different from the bees from group VII (Slovenia). Of analyzed measures of the hind legs of bees, only the length of the femur and width of basitarsus showed statistical significance. Femur length ranged from 2.547 to 2.655 mm, and corresponds with investigations from *Nedić (2009).* Considering this trait, bees from northern Serbia (group II) were very significantly different (P <0.01) than bees from southern Serbia (group V). Basitarsus width ranged from 1.145 mm in bees from Slovenia (VII) to 1.212 mm in bees from southern Serbia (V) and this difference was statistically highly significant (P <0.01). Results for this feature are consistent with research of *Jevtić (2007)* where the maximum basitarsus width was measured in Rasina ecotype in Central Serbia (1.16 mm), and lowest in the Kopaonik ecotypes (1.05 mm). *Georgijev (2006)* reported a slightly higher range for the basitarsus width (1.04 to 1:37 mm) in the examined bees from eastern Serbia.

Table 1. Means and standard deviation (Sd) of selected measures for the bee samples.
Sizes of angles are given in degree (°), size of other morphometrical characters are given in millimeter (mm).

Char.	Origin of Samples (locations)							Average
	Središte	Kljajićevo	B.Bašta	Aleksandr.	Babušnica	Kozarevo	Slovenia	
	I	II	III	IV	V	VI	VII	
A4 (°)	29.15±1.25 ab*	29.85±1.58 a	27.64±1.39 b	29.74±0.98 a	29.00±1,94 ab	29.43±2.12 a	29.74±1.52 a	29.22±1.69
B4 (°)	110.56±5.98 a	111.79±5.69 a	113.37±5.42 a	113.00±3.75 a	112.85±5.38 a	109.18±4.51 a	110.42±4.28 a	111.59±5.13
D7 (°)	98.12±2.46 a	98.79±3.72 a	96.75±3.33 a	98.96±3.14 a	98.22±3.01 a	96.04±3.19 a	98.42±2.00 a	97.90±3.11
E9 (°)	24.32±2.48 a	24.45±1.64 a	23.08±1.59 a	24.07±1.38 a	23.42±1.86 a	23.69±1.41 a	23.68±1.80 a	23.82±1.78
L13 (°)	15.54±1.20 a	14.04±1.05 a	15.02±1.44 a	14.40±1.42 a	15.33±1.06 a	14.24±1.51 a	14.64±1.33 a	14.75±1.37
J10 (°)	54.65±4.51 ab	52.51±2.80 b	55.05±3.04 ab	55.27±3.09 ab	56.83±6.38 a	57.30±2.59 a	53.62±2.55 ab	55.03±4.00
J16 (°)	93.82±3.51 a	90.75±4.45 ab	93.98±2.87 a	88.49±4.46 b	90.93±3.62 ab	91.08±2.27 ab	92.81±2.91 a	91.69±3.88
N23 (°)	94.55±2.66 ab	92.07±3.39 b	95.39±3.96 ab	94.22±3.71 ab	93.49±3.81 ab	97.11±3.56 a	95.63±2.39 ab	94.64±3.63
K19 (°)	78.24±2.34 a	78.42±2.94 a	78.13±2.64 a	79.09±3.44 a	78.03±2.17 a	76.90±2.50 a	79.33±2.16 a	78.31±2.66
G18 (°)	92.57±2.41	91.73±4.46	90.13±3.29	89.99±2.63	91.00±4.82	91.38±3.03	92.96±2.30	91.39±3.47

	a	a	a	a	a	a	a	
O26 (°)	39.06±2.68 a	36.43±3.66 a	35.55±3.11 a	34.89±3.22 a	36.41±2.55 a	36.43±4.37 a	37.38±2.51 a	36.59±3.36
W1 (°)	133.95±3.01 bc	136.26±4.08 abc	132.33±3.95 c	137.17±2.71 ab	138.22±5.19 a	133.44±4.30 bc	135.16±3.47 abc	135.22±4.26
W2 (°)	71.24±3.39 a	71.95±3.49 a	74.74±3.67 a	67.32±2.33 b	71.51±4.92 a	73.25±3.17 a	71.48±3.20 a	71.64±4.03
W3 (°)	26.21±1.00 a	26.22±1.11 a	25.82±1.05 a	25.91±1.63 a	26.67±0.94 a	27.15±1.47 a	26.23±1.75 a	26.31±1.34
Cub a (mm)	0.570±0.038 a	0.568±0.040 a	0.589±0.030 a	0.545±0.042 a	0.582±0.042 a	0.587±0.055 a	0.596±0.037 a	0.576±0.043
Cub b (mm)	0.218±0.020 b	0.219±0.044 b	0.246±0.025 a	0.238±0.016 ab	0.231±0.019 ab	0.251±0.020 a	0.232±0.023 ab	0.234±0.028
FWL (mm)	9.014±0.242 d	9.049±0.163 cd	9.093±0.109 bcd	9.227±0.158 ab	9.199±0.141 abc	9.229±0.137 a	9.319±0.126 a	9.162±0.185
FWW (mm)	3.165±0.081 c	3.202±0.068 bc	3.161±0.065 c	3.244±0.052 ab	3.297±0.055 a	3.280±0.094 a	3.311±0.054 a	3.237±0.088
HWL (mm)	6.401±0.217 b	6.416±0.121 a	6.420±0.170 a	6.587±0.155 a	6.502±0.139 a	6.568±0.241 a	6.574±0.105 a	6.495±0.183
HWW (mm)	1.836±0.049 c	1.884±0.052 bc	1.858±0.080 bc	1.907±0.041 b	1.919±0.089 ab	1.939±0.051 ab	1.982±0.049 a	1.902±0.077
TEW (mm)	4.702±0.139 bc	4.529±0.164 d	4.833±0.159 ab	4.573±0.101 cd	4.701±0.188 bc	4.789±0.171 ab	4.881±0.118 a	4.716±0.191
TEL (mm)	2.212±0.059 bc	2.132±0.058 d	2.183±0.072 bcd	2.166±0.056 cd	2.204±0.067 bc	2.242±0.058 ab	2.292±0.062 a	2.204±0.077
FEL (mm)	2.636±0.062 ab	2.547±0.069 c	2.587±0.059 bc	2.629±0.048 ab	2.655±0.046 a	2.589±0.030 bc	2.624±0.073 ab	2.609±0.065
TIL (mm)	3.193±0.093 a	3.196±0.093 a	3.176±0.055 a	3.186±0.086 a	3.263±0.059 a	3.161±0.070 a	3.215±0.094 a	3.199±0.088
BAL (mm)	2.052±0.074 a	2.047±0.061 a	2.026±0.096 a	2.017±0.065 a	2.073±0.080 a	2.030±0.059 a	2.080±0.063 a	2.046±0.068
BAW (mm)	1.185±0.037 abc	1.175±0.045 abc	1.199±0.049 ab	1.162±0.029 bc	1.212±0.054 a	1.179±0.055 abc	1.145±0.037 c	1.179±0.048

*Means for the same characteristics followed by different letters within locations are significantly different (P<0.01) according to variance analysis followed by Duncan´s Studentized multiple range tests.

To differentiate the races and, also, for the characterization of subpopulations within each race, multivariate discriminant analysis proved to be very useful *(Kauhausen-Keller and Keller, 1994)*. In our study, the analysis contained a total of 26 steps, exactly as many characters were adopted in the analysis of 7 groups of honey bees. The results of multivariate discriminant analyses showed that significant differences existed between the seven groups of bees (Wilk´s λ = 0.00586, P<0.00001). The 1st axis (CV 1) explained 34.2%, the 2nd (CV 2) 29.2% and the 3rd axis (CV 3) 12.4% of the total variation. Thus 75.8% of the total variation could be explained by first three canonical variants.

Based on this analysis, the Figure 1 shows the distribution of the centroids of the analyzed bee samples According to the first discriminant axis, which explains most of the total variability, centroids of group III and V are located in the first quadrant,

and the bees group VI (Southwestern Serbia) and in the group VII (Slovenia) centroids are located in the second quadrant. Bees of group IV are separated in the third quadrant, while the centroids of bee groups I and II are located together in the fourth quadrant. A similar arrangement of centroids is represented by the second discriminant axis. Angles of the front and hind wing A4, J10, J16, W1 and W2, length of cubital vein b, FWL, FWW, HWL, HWW, TEL and BAW contributed greatly to the separation of the groups. Based on the canonical discriminant analysis *Stevanović (2002)* has separated three ecotypes. Banat and Sjenica-Pešter ecotype were separated on the first canonical axis by the angles G18, E9 and B4, while on the second canonical axis are separated Timok ecotype on one side and the Banat and Sjenica-Pešter ecotype and the other due to differences in the length of the front wings, femur length and the size of the angle G18.

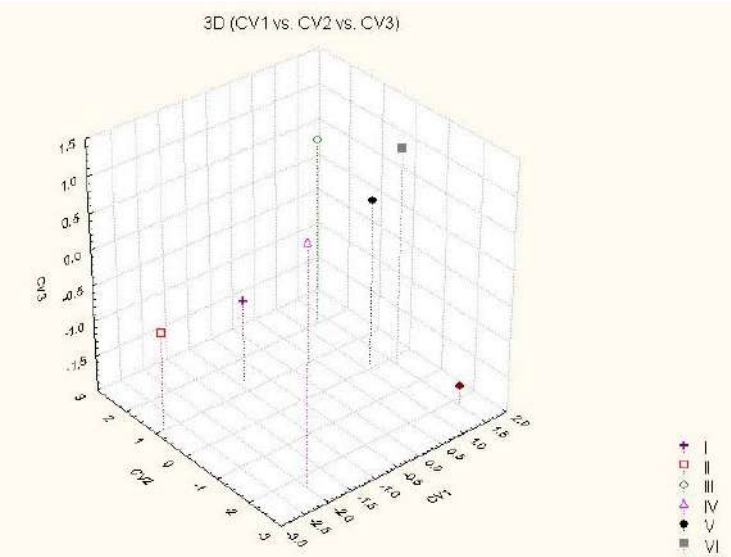

Figure 1. Three dimensional view of the distribution of honeybee groups centroids by sites

Under conditions in which the bees are bred in Serbia, migratory beekeeping is widespread. This phenomenon is present from mid May and flowering of false acacia, followed by migration of bee colonies during the basswood pasture in June, after which the bees are moved in early July to the plains in the north (Vojvodina) to the sunflower pasture. In early June, the trade of swarms, and with them the young queens, is the most intense. The second period of queen substitution happens during the sunflower pasture. Queen bee market in Serbia is free, and trading of queens can be done both by the selection centers and by beekeepers. However, the capacity of selection centers for the production of quality breeding queens are at

about 25000-30000 queens a year and the number of bee colonies in Serbia at about 350000. Since the production capacity for the production of selected queens are limited, and that the customer's choice of where to buy the queens is free, it is expected that, beside the biotopes in which bees live and the natural influence of neighboring subspecies *Apis mellifera macedonica*, there is introgression of traits of Carniolan bee in Serbia caused by human activity.

Based on the analyzed angles measured in the front and the hind wing, some groups of bees from Serbia (table 1) more closely correspond to the values *Kauhausen-Keller and Keller (1994)* reported the breeding lines of *A. m. carnica* from Kirchhain than to the reference for *Carnica* from the data bank, presented in round and square brackets, respectively: A4 (29.7^o)[28.3^o], B4 (109.1^o) [111.8^o], D7 (97.5^o) [99.0^o], E9 (24^o) [22.7^o], G18 (92^o) [12.3^o], J10 (53.4^o) [52.7^o], J16 (92.9^o) [95.9], K19 (78.0^o) [79.0^o], L13 (13.7^o) [12.3^o], N23 (93.0^o) [93.9^o], O26 (35.2^o) [37.8^o], W1 (142.7^o) [140.7^o], W2 (71.9^o) [70.7^o], W3 (27.6^o) [26.2^o]. However, *Ruttner (1988)* previously studied wing characters E9 (23.12±2.08), G18 (93.10±3.33), J10 (52.20±3.45) and L13 (12.48±2.96) and in relation to those values, our results are different for angles G18, J10 and L13.

Among the analyzed bees from Serbia, the most similar by the examined traits were group I and III. Those two groups differed ($P < 0.01$) only in the length of cubital vein b. Although the bees were sampled from remote sites, their similarity can be explained by migration and the human factor, because the bees from group III were moved to sunflower pasture in the native area of group I (Banat). Great similarity were found between bees of groups V and VI from southern Serbia, and significant difference ($P < 0.01$) was found only in size of characters W1 and FEL. The greatest differences were observed between groups II (northwest Serbia) and VI (southwestern Serbia) in characters J10, N23, CUB b, FWL, FWW, TEW and TEL. Bees of group II had short and narrow front wings (9.049 ± 0.163 mm and 3.202 ± 0.068). *Mladenović and Radoš (2008)* reported that the yellow bees from Vojvodina have the average length and width of the front wing is 9.05 and 3.08 mm, respectively. *Georgijev (2006)* stated that there is variation from 9.28 to 9.79 mm in the length of the front wings in bees from eastern Serbia. *Nedić (2009)* indicated that the front wing width is 3.23 mm in bees from Knjaževac (east Serbia) and 3.34 mm in bees from Vranje (south Serbia).

The results of the examined characters (table I) show that between bees from group VI (Kozarevo), and bees from group VII (Slovenia) there are no statistically significant differences ($P > 0.01$). In discriminant analysis, centroids of the two groups are placed together in the second quadrant. A sample of bees in the group VI comes from the stationary apiaries from southwest Serbia where the foreign biological material was not introduced and it represents an isolated biotope where the bees are reared in the traditional indigenous form.

By the studied characters, sampled bees from southern Serbia (group V) are very close to group VII because they differ (P <0.01) only in the width and length of tergite and in the basitarsus width. In relation to the group VII, most bees varied was group II (from the northwest Serbia), which was mainly influenced by a very small length and width of the front and hind wings, small length and width of the third tergite and femur length. *Dražić et al. (1998),* in Croatia, on the basis of similar morphological characters, indicated the great diversity of bees in the Mediterranean area in relation to bees native to the mountain, and Pannonian regions, between which the differences were not pronounced.

Conclusion

Research has shown that in the addition of angles A4, J10, J16, W1 and W2, characters CUB b, FWL, FWW, HWL, HWW, TEL, BAW contributed the most to the differentiation of the honey bee groups. Comparisons of individual groups of bees from Serbia and samples of bees from Slovenia show close morphological relation because *Apis mellifera carnica* is native to the region. Within Serbia, the results suggest that by the characteristics of morphological characters, there are groups of bees that were allocated on very distant geographical locations, i.e. one in the north of Vojvodina and the other in the south of the country. Although the results support this view, morphometric characters are sensitive to the selection impact and changes in the environment (*Avise, 1987*) and therefore the research should be extended by analysis of molecular markers. Determined variability provides an opportunity for further promotion and selection of bees that meet the criteria for the morphometric traits stipulated for the *Apis mellifera carnica*.

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Morfološka karakterizacija medonosne pčele (*Apis mellifera L.*) sa različitih lokaliteta Srbije

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Rezime

Cilj ovog istraživanja je bio da se analizira veličina 26 morfometrijskih karatktera uzoraka pčela sakupljnih sa šest različitih lokacija na teritoriji Srbije i uporediti ih sa vrednostima izmerenih karaktera na referentnom uzorku medonosnih pčela iz Slovenije. Diferencijaciji ispitivanih uzoraka pčela najviše je doprinela analiza uglova na prednjem i zadnjem krilu A4, J10, J16, W1 i W2, i karakteri CUB b, FWL, FWW, HWL, HWW, TEL, BAW. Poređenja analiziranih grupa pčela iz Srbije i uzoraka pčela iz Slovenije pokazuju blisku morfološku vezu, jer je podvrsta pčela *Apis mellifera carnica* nativna u regionu. Rezultati ispitivanja pčela unutar Srbije ukazuju da postoje grupe pčela koje su izdvojene na vrlo udaljenim geografskim lokacijama, jedne na severu u Vojvodini i druge na jugu zemlje gde je prisutan prirodni uticaj susedne podvrste pčela (*Apis mellifera macedonica*). Utvrđena varijabilnost pruža priliku za nastavak istraživanja i izbor pčela koje ispunjavaju kriterijume za morfometrijske osobine karakteristične za *Apis mellifera carnica*.

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QUALITY PARAMETERS AND AUTHENTICITY OF ROYAL JELLY

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Review paper

Abstract: Due to specific chemical composition, royal jelly is one of the most valuable bee products used by man since ancient times. Because of the presence of physiologically active compounds that have positive effects on human health, this bee product is primarily used in the pharmaceutical industry, cosmetics and production of healthy food. The present paper aimed to discuss parameters of quality and authenticity of royal jelly, which must satisfy certain national and international standards, in order to be competitive product in the food market. Royal jelly is a creamy yellowish substance produced by the cephalic glands of worker bees (*Apis mellifera* L.) in certain developmental stage, for the purpose of larvae feeding. Considering that this is a highly sought product in the market, whose production is constantly increasing, there is a need for developing new methods of verification of its quality and authenticity. The most important criterion for establishing the quality and authenticity of royal jelly is the determination of 10-hydroxy-2-decenoic acid (10-HDA), whose concentration varies widely, depending on a number of factors. The chemical composition of royal jelly is changed if stored for a long time, or due to the lyophilization. Besides 10-HDA analysis, in recent years, variety of methods for determining the authenticity of royal jelly has been developed, but so far, none of them can be singled out as the most reliable. Improving methods for quality and authenticity assessment, in accordance with established national and international standards, remains a challenge for science.

Key words: royal jelly, quality, geographical authenticity

Introduction

Royal jelly is formed by activity of hypopharingeal glands of worker bees and it represents food of queen bee and larvae, up to three days of age *(Garcia-Amoedo et al., 2007)*. In addition to its use in traditional medicine, many scientific

experiments confirmed positive effect of royal jelly on human health *(Nakaya et al., 2007; Kohno et al., 2004; Morita et al., 2012; Park et al., 2011)*, causing that this product has become very desirable and highly appreciated in the market. Royal jelly is a creamy yellowish substance, which is used fresh or dried (lyophilized), mainly as a dietary supplement while recovering from an illness or during severe stress. The chemical composition of this product is complex, consisting of proteins, amino acids, sugars, lipids and vitamins *(Bărnuţiu et al., 2011)*. In the industry, royal jelly is primarily used for cosmetics, medicines or food products *(Daniele and Casabianca, 2012)*. Due to the high demands in the market, it has reached a high price and became the subject of adulteration. Substances used for adulteration can not be detected by organoleptic methods, and include adding of corn starch, yogurt, egg whites, condensed milk with propolis, unripe bananas and water *(Garcia-Amoedo et al., 2007)*. Today, the largest amount of royal jelly is produced in China, so thousands of tons of this product is placed on the world market per year, at affordable prices *(Daniele and Casabianca, 2012)*. While some countries such as Brazil, Bulgaria, Japan and Switzerland have developed national standards for royal jelly quality, international standards for quality and authenticity of this product do not exist yet *(Ramadan et al., 2012)*. Due to the liberalization of the global food market and increasingly strong competition of producers in the conquest of new markets, it is necessary to develop international standards, which would regulate the issue of royal jelly quality and authenticity, to achieve greater security primarily in terms of food safety and origin.

The Quality of Royal Jelly

According to *Grunert (2005)*, an objective assessment of the quality of products includes physical-chemical characteristics of the product, which are analyzed by experts, and the subjective quality impression about the product, indicated by the average consumer obtained when eating. Scientific research related to the impression of consumers has not been conducted yet but many studies has been devoted to the physical, chemical and sensory properties of this product. Physico-chemical properties of this natural substance, which are most frequently analyzed are as follows: sugar composition, moisture, proteins and 10-HDA *(Daniele and Casabianca, 2012)*. Quality parameters include content and composition of sugars, water and proteins content, as well as sensory properties (smell, taste, color), while the freshness of royal jelly is determined based on the concentration of the 10-HDA, glucose oxidase and values of furosine. More than 80% of sugars are fructose and glucose, and in addition to that, there are 12 more sugars present in small amounts - galactose, mannitol, maltose, maltulose, turanose, trehalose, palatinose, isomaltose, gentiobiose, melezitose, maltotriose,

and erloza *(Daniele and Casabianca, 2012)*. The same authors state that these sugars can serve as markers of geographical authenticity of the product. The water content is quite constant and no greater than 60%. The largest part of dry matter is made of proteins, wherein there are considerable differences in protein content between fresh and lyophilized royal jelly. Namely, according to *Popescu et al. (2009*), the fresh royal jelly has lower protein content (7.7 mg/ml) compared to a lyophilized royal jelly (19.8 mg/ml). *Liming et al. (2009)* showed that the average content of total amino acids amounts to 111.27 mg/g, wherein, the most abundant is the aspartic acid with concentration of approximately 21.04 mg/g. Significantly smaller amount of glutamic acid (12.29 mg/g), lysine (10.05 mg/g) and leucine (9.53 mg/g) has been found. The same survey showed that with the increase of temperature and storage time, the concentration of glutamine, glycine and leucine slightly increased while the concentration of lysine, histidine and methionine decreased. Due to this fact, the same authors suggested that amino acids could be parameters for quality evaluation. Sensory properties, which also indicate quality, include: colour, aroma, taste, odour and viscosity. The color varies from white to pale-yellow (when stored for long period it changes colour to yellow), and the odour and taste are sour *(Popescu et al., 2008)*. Viscosity is an important parameter of quality and varies depending on the water content and the storage conditions. According to *Ramadan et al. (2012)*, the increase in viscosity is related to the increase of nitrogen compounds insoluble in water and dicrease of soluble nitrogen and free fatty acids, which occurs when preserving royal jelly at room temperature. The most analyzed acid is 10-hydroxy-2-decenoic acid (10-HDA) indicating freshness and authenticity of royal jelly if it is contained over 1.8% *(Antinelli et al., 2003)*. By studying qualitative and quantitative properties of this substance, *Antinelli et al. (2003)* concluded that, at room temperature, there is a rapid degradation of the 10-HDA after the third month, which can be detected by sensory observation, whereas in samples stored at very low temperatures this was not the case, or the content of this acid did not significantly decreased during storage. Hence, in practice, royal jelly is considered fresh if not older than 3 months *(Antinelli et al., 2003)*. Degradation process of 10-HDA stops at -18°C, whereas at temperatures above 4°C, 20-50% of its content is lost. However, the same authors stated that the determination of 10-HDA could not be used as a reliable indicator of freshness. According to *Barnutiu et al. (2011)*, the concentration of 10-HDA in royal jelly is about 1.5-2.0%, and this parameter is an internationally recognized standard for determining royal jelly quality, which directly impacts the price in the international market. According to *Sabatini et al. (2009)*, the concentration of the enzyme glucose oxidase can indicate the freshness of royal jelly. Namely, time and storage temperature affect this enzyme content, whereby at the temperature of 20°C after one month, its concentration is significantly reduced, and a year after,

enzyme is completely degraded. Another potential indicator of royal jelly freshness is furosine *(Marconi et al., 2002; Messia et al., 2005)*. In a fresh royal jelly, furosine content is low (0-10 mg/100 g of protein), but it is significantly increased with prolonged storage at higher temperature. Furosin content can be increased up to 500 mg/100g of protein after 18 months of storage at room temperature, or up to 50 mg/100 g of protein after 18 months of storage at 4°C *(Sabatini et al., 2009)*.

Adulteration of royal yelly

Royal jelly is usually adulterated by adding yogurt, egg whites, water and corn starch, which can be detected by changes of the physico-chemical characteristics. Namely, adding more than 25% of these substances in the royal jelly, leads to increase in moisture, followed by lipid, protein, and 10-HAD reduction, as well as insolubility in basic medium. Samples adulterated by mentioned substances in concentrations of about 10% can be detected by a slight increase in moisture, reduce in lipids, proteins and 10-HDA, as well insolubility in basic medium. Royal jelly adulterated by condensed milk in an amount of over 10% can be detected based on the increase of lipids and decrease of humidity, protein and 10-HDA content *(Garcia-Amoedo et al., 2007)*. Adulterated royal jelly is detected by routine analysis of quality and authenticity. To determine geographical authenticity, the easiest and the most accessible method is to ascertain the pollen spectra in this bee product, which requires the engagement of highly skilled people who know not only the pollen morphology, but also the apiflora and vegetation in the area of royal jelly origin. In addition to pollen analysis, other methods for determining the quality and authenticity of royal jelly were developed (Table 1).

Table 1. Some methods for determination of royal jelly quality and authenticity (*Sabatini et al., 2009*)

Parameter	Method
Water content	Determined by freeze-drying (*Messia et al., 2005*), Karl Fischer (*Ferioli et al., 2007*), vacuum oven, dessication (*Garcia-Amoedo and Almeida-Muradian, 2002, 2007*)
Total protein	Nitrogen determined with the Kjeldahl method (*Lercker et al., 1992-93*), free amino acids determined by ion chromatography (*Boselli et al., 2003*)
Carbohydrates	Determined by gas (*Lercker et al., 1992-93*) or liquid chromatographies (*Sesta, 2006*)
Lipids	Determined as free and total organic acids by gas chromatography (*Lercker et al., 1992-93*) or as total lipids, by solvent extraction (*Karaali et al., 1988*)
10-HDA	Determined by HPLC (*Bloodworth i sar., 1995; Genc and Aslan, 1999*)
Minerals	Determined by atomic absorption (*Benfenati et al., 1986*)
Acidity	Titration method (*Serra-Bonvehi, 1992*)
Sediment analysis	Microscopical analysis (*Ricciardelli D'Albore, 1986*)
Furosine	(*Marconi et al., 2002*)

Also, in addition to developing methods, it is necessary to establish international standards for determination of royal jelly quality that comes to market (Table 2).

Table 2. Proposed criteria for royal jelly composition (*Bogdanov and Gallmann, 2008*)

Royal jelly	Fresh(*)	Lyophilized
Water	60-70	<5
Lipids	3-8 (4-8)	8-19
10-HAD	>1,4 (1,4-6)	>3,5
Proteins	9-18	27-41
Fructose-glucose-sucrose	7-18 (11-23)	-
Fructose	3-13	-
Glucose	4-8	-
Sucrose	0,5-2,0	-
Ash	0,8-3,0	2-5
pH	3,4-4,5 (3,5-4,1)	3,4-4,5
Acidity	3,0-6,0	-
Furosine	<50* (-)	-

In its bylaws, the Republic of Serbia did not especially defined royal jelly quality in the market, but in the Regulations on the quality of the honey, products based on honey and other bee products, the term of royal jelly is defined, as well as form of sale (original form, stabilized or lyophilized), way of storage (in the dark, hermetically sealed glass vessels at temperatures ranging from -6 to -4^{o}C) as well as the basic requirements in terms of its physical and chemical characteristics. This

implies that royal jelly contains no more than 70% of water and 30% of dry matter and at least 11% of proteins *(Službeni list SCG br. 448/1, 2003)*.

Royal Jelly authenticity

The authenticity of food products is linked to the area of their origin, and that area is characterized by certain environmental conditions as well as mode of production. Raw materials and food products at the market with known geographic origin are valued more because they are considered of higher quality and healthier. The reason for this is not a small number of adulterated products, for example wine, olive oil and dairy products, which is very detrimental to the economy and reputation of the country of origin *(Anklam, 2001)*. The authenticity of royal jelly production, is routinely estimated by concentration of 10-HDA *(Bogdanov, 2008)*, however, this method can not be used for determining the geographical origin. Methods for determining the geographical authenticity of royal jelly include polen spectra identification and determination of stable isotopes of C and N. The principle of determining the botanical origin of honey, based on the type and percentage of pollen grains, also can be applied in the case of royal jelly. Research of *Dimou et al. (2007)* have shown that the palynological analysis of royal jelly can be used as a reliable indicator of geographic origin, considering that in the samples analyzed, a correlation between the pollen spectra and vegetation in the area of royal jelly origin exists. Since the analysis of stable isotopes of carbon can be used to determine the honey authenticity, in terms of its possible adulteration with sugar of C4 plants, similar principle can be applied in the case of determining the authenticity of royal jelly production. Namely, by analysing the stable isotopes of C and N, *Stocker et al. (2006)*, concluded that on the basis of this ratio, genuine from adulterated jelly can be distinguished, but it is necessary to continue research related to this issue, as in the case of determining honey geographical authenticity. Namely, for the determination of the region of origin, it is necessary also to analyze isotopes of other elements, such as strontium, sulfur, oxygen, and hydrogen.

Conclusion

Due to the its positive impact on human health, royal jelly is a bee product that is increasingly demanded on European and world markets. However, it is necessary to reliably determine the quality and authenticity of royal jelly that is sold to consumers. This could be performed by analyzing the following parameters: the contents of 10-HDA, sugar, water, proteins and fats as well as on the basis of pollen analysis. Methods for accurately determining adulterated royal

jelly and its geographical origin are still developing and there are attempts to establish international standards regarding this issue.

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Parametri kvaliteta i autentičnosti matičnog mleča

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Rezime

Zbog specifičnog hemijskog sastava, matični mleč predstavlja jedan od najdragocenijih pčelinjih proizvoda koje čovek koristi od davnina. Zbog utvrđenih pozitivnih efekata na zdravlje ljudi, usled prisustva fiziološki aktivnih jedinjenja, ova se materija koristi prevashodno u farmaceutskoj industriji, kozmetici i proizvodnji tzv. zdrave hrane. Cilj rada je predstavljanje parametara kvaliteta i autentičnosti matičnog mleča, koji moraju biti u skladu sa nacionalnim i međunarodnim standardima kako bi se mleč plasirao na tržište hrane. Matični mleč je žućkasta kremasta supstanca koja nastaje radom cefaličnih žlezda pčela radilica (*Apis mellifera* L.) u određenom stadijumu njihovog razvića radi ishrane larvi. S obzirom da je u pitanju proizvod koji je veoma tražen na tržištu, i čija se proizvodnja konstantno povećava, javila se neophodnost razvijanja novih i usavršavanja postojećih metoda pomoću kojih je moguće ustanoviti kvalitet mleča i njegovu autentičnost. Najvažniji kriterijum za procenu kvaliteta i autentičnosti ovog pčelinjeg proizvoda je određivanje sadržaja 10-hidroksi-2-decenoične kiseline (10-HDA), čija koncentracija veoma varira u zavisnosti od većeg broja faktora. Naime, hemijski sastav mleča se menja pri dužem čuvanju kao i pri liofilizaciji. Osim određivanja sadržaja 10-HDA, poslednjih godina se unapređuju metode za utvrđivanje autentičnosti mleča ali za sada, nijedna se ne može izdvojiti kao najbolja. Usavršavanje ovih metoda uz uspostavljanje međunarodnih i nacionalnih standarda kvaliteta i autentičnosti, ostaje izazov za nauku.

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CORN SILAGE PROCESSING SCORE (CSPS)

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Original scientific paper

Abstract: The Corn Silage Processing Score (CSPS) was developed (USDA Forage Research Center) as a tool to define adequacy of kernel processing by forage harvesters. Authors investigated Corn Silage Processing Score (CSPS) of 222 corn silage samples collected from Hungary in 2013 and 2014. Corn silage processing score (CSPS) was determined on dried samples with Ro-Tap Sieve shaker according to Ferreira and Mertens (2005). The ratio of inadequately processed corn silages were rather high in Hungary (2013: 28% vs 2014: 23%). Although, 65% of the corn silage samples were adequate from processing point of view (2013 and 214). According to our results, 7% (2013) and 12% (2014) of the analysed corn silages were in the optimal quality range. Inadequate corn processing can cause serious losses due to the lower real starch digestibility. Therefore it is recommended to introduce *corn silage processing quality control* on the dairy farms during the corn harvest period

Keywords: corn processing, corn silage, CSPS

Introduction

The Corn Silage Processing Score (CSPS) was developed (USDA Forage Research Center) as a tool to define adequacy of kernel processing by forage harvesters. The CSPS is the percentage of starch passing through the coarse 4.75 mm screen (Ferreira and Mertens, 2005). Processing of corn silage may improve dry matter intake, starch digestion, and lactation performance (Bal et al., 2000 a,b). Dry matter intake (25.9 vs. 25.3 kg/d) and milk (46.0 vs. 44.8 kg/ d) and fat (1.42 vs. 1.35 kg/d) yields were higher for the processed corn silage treatments (at a 1 mm roll clearance) compared with the control corn silage (Bal et al., 2000a). Kozakai et al. (2007) have found more rapid and greater colonization of the processed compared to unprocessed silage, by the rumen bacteria which facilitates ruminal digestion and fermentation. Authors investigated Corn Silage Processing Score (CSPS) of 221 corn silage samples collected from Hungary in 2013 and 2014.

Material and Methods

Samples from different areas of Hungary collected by farmers during 2013 (n.147) and 2014 (n. 74) were assessed. Samples were dried (70 °C, 8 hours) according to method EN ISO 6496:1993. Starch content was determined by Near-InfraRed Spectroscopy. Spectra were determined according to the guidelines of NEN-EN-ISO 12099 (Q-Interline Quant FT-NIR analyser). Reference starch method: NEN-EN-ISO 15914. Corn silage processing score (CSPS) was determined on dried samples with Ro-Tap Sieve shaker according to Ferreira and Mertens (2005). Modified starch digestibility, modified digestible starch and modified net energy content according to CSPS value were calculated based on Schwab et al. (2003).

Results and discussion

Results and distribution of the quality are given in Table 1.

Table 1 Distribution and evaluation of Corn Silage Processing Score of fresh whole corn crop (chopped) and corn silage

Quality distribution according to CSPS value		Harvest 2013		Harvest 2014	
		No. sample	%	No. sample	%
<50	inadequate	41	28	17	23
50-70	adequate	96	65	49	65
>70	optimal	10	7	9	12

The ratio of inadequately processed corn silages were rather high (2013: 28% vs 2014: 23%). Although, 65% of the corn silage samples were adequate from processing point of view (2013 and 214). According to our results, 7% (2013) and 12% (2014) of the analysed corn silages were in the optimal quality range.

Detailed results of starch digestibility based on the actual CSPS value and calculated losses can be found in Table 2. The CSPS mean values of corn silages derived from 2013 were adequate, but not optimal causing losses of 41 g/kg DM starch and 0.29 MJ/kg DM net energy compared to the original laboratory results (without CSPC) due to undigested starch in inadequately processed corn silages (seed size: >4.75 mm). The losses (based on undigestible starch content) were equivalent to 14 ha corn grain, as average (2013). The CSPS value of 58% (2014) were equivalent to 17.3 ha corn grain (based on undigested starch content).

Table 2 Corn Silage Processing Score, starch digestibility and net energy content of corn silages

	CSPS	Fresh forage and corn silage			Corn silage	
		Original starch	Modified starch digestibility	Modified digestible starch	Original NEl	Modified NEl
	%	g/kg DM	%	g/kg DM	MJ/kg DM	MJ/kg DM
Harvest 2013, n= 147						
Average	55	286	86	245	6.32	6.03
St. deviation	11	55	9	50	0.23	0.26
Starch losses					41 g/kg DM starch	
NEl losses					0.29 MJ/kg DM	
Milk losses (energy equvivalent, 7kg DMI silage, 3 MJ NEl					0.69 kg milk/day/cow	
Losses (starch equvivalent 1 year, 500 dairy cow, 7 kg DMI					14.0 ha corn seed	
Harvest 2014, n= 75						
Average	58	357	87	306	6.51	6.13
St. deviation	10	62	9	46	0.21	0.25
Starch losses					51 g/kg DM starch	
NEl losses					0.38 MJ/kg DM	
Losses (energy equvivalent, 7kg DMI silage, 3 MJ NEl /kg					0.93 kg milk/day/cow	
Losses (starch equvivalent 1 year, 500 dairy cow, 7 kg DMI					17.3 ha corn seed	

Conclusion

Inadequate corn processing can cause serious losses due to the lower real starch digestibility. It is recommended to introduce *corn silage processing quality control* on the dairy farms during the corn harvest period, to improve the management and technical background in order to reduce the financial losses caused by inadequate CSPS values.

Ocena obrade silaže kukuruza - Corn silage processing score (CSPS)

Sz.Orosz, L. Dégen, O. Petrák, Zs. Kövesdi

Rezime

Ocena obrade kukuruzne silaže - The Corn Silage Processing Score (CSPS) je razvijen (Istraživački centar za krmno bilje Ministarstvo poljoprivrde SAD - USDA Forage Research Center) kao sredstvo da se definišu adekvatnost obrade zrna od strane kombajna za žetvu. Autori su ispitali Ocenu obrade kukuruzne silaže (CSPS) od 222 kukuruzne silaže uzoraka sakupljenih u Mađarskoj u 2013. i 2014. Ocena obrade kukuruzne silaže (CSPS) je rađena na sušenim uzorcima sa Ro-Tap Sieve šejkerom prema metodi Ferreira i Mertens (2005). Procenat/udeo nedovoljno obrađenih kukuruznih silaža je bio prilično visok u Mađarskoj (2013: 28% u odnosu na 2014: 23%), iako je 65% uzoraka kukuruzne silaže bilo adekvatno sa tačke gledišta prerade (2013 i 214). Prema našim rezultatima, 7% (2013) i 12% (2014) analiziranih silaža kukuruza su u optimalnom opsegu kvaliteta. Nedovoljna obrada kukuruza može da izazove ozbiljne gubitke zbog niže realne svarljivosti skroba. Zbog toga se preporučuje da se uvede kontrolu kvaliteta obrade kukuruzne silaže na farmama u toku perioda žetve kukuruza.

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QUALITY OF WHOLE-PLANT CORN SILAGE ON FAMILY FARMS

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Original scientific paper

Abstract: Whole-plant corn silage is a starting point for economical and modern animal husbandry. It is a quality green feed replacement and the most important cattle feed. The quality of whole-plant corn silage is of great importance in milk production. Continuous monitoring of silage quality is an important part of feeding programmes. The aim of this study was to use chemical and microbiological analyses to determine the quality of whole-plant corn silage produced on family farms. The analysed samples contained, on average, 66.37% moisture (58.79-72.01%), 33.63% dry matter (27.99-41.21%), 2.07% crude protein (1.07-3.06%), 1.26% crude fat (0.72 -1.84%) and 8.09% crude cellulose (5.72-9.98%). The average pH value, taken as an indicator of the quality of fermentation, was 3.90 (3.50-4.16). Total volatile fatty acid comprised 1.87% (0.67-2.47%) lactic acid, 0.68% acetic acid (12.20-1.52%) and 0.02% (0-0.27%) butyric acid. The average proportions of lactic, acetic and butyric acids in the silage were 72.80% (33.3-88.81%), 26.45% (9.35-66.67%) and 0.75% (0-10.16%). The authors determined silage quality using the Flieg score. The samples from 12 farms were graded as very good, while the silage from the other farms was evaluated as good (4 farms) and satisfactory (2 farms). The analysis of microbiological quality showed that the samples on average contained 405,556 (10,000-3,000,000) of silage bacteria and 7,000 (0-80,000) of mould. Sulphate-reducing Clostridia and Salmonella were not identified. The samples matched quality requirements prescribed by the rulebook.

Key words: silage, analysis, quality, family farms

Introduction

Silage is a starting point for economical and modern animal husbandry. It is a quality green feed replacement and the most important cattle feed. Modern

technologies of ensiling whole corn plants aim at obtaining high quality silage with as higher nutrient level as possible, by using methods that enable maximum preservation and a high level of utilization of nutritive values (*Johnson et al., 1999*). Whole-plant corn silage is one of the most important nutrients in the diet of dairy cows (*Đorđević et al., 2011*).

Corn gives high yields of green biomass. Its dry matter contains relatively high energy levels it is well-consumed by cows. In countries with intensive cattle breeding, conserved bulk feed is used throughout the year, combined with concentrates. Silage is one of the main forms of conserved bulk feed. An essential characteristic of whole-plant corn silage is digestibility, which affects the utilisation of nutrients. An important factor that determines the digestibility is the maturity of silage corn (*Ferraretto and Shaver, 2012*; *Akins and Shaver, 2014*).

Whole-plant corn silage can be used during winter, or throughout the year (*Der Bedrosian et al, 2012*). It is characterized by good taste and it is an important nutrient for preparing totally mixed rations (*Neylon and Kung, 2003*). The quality of silage depends on the botanical classification of plants, stage if plant development, growing cycle, ensiling conditions, silo facilities, ensiling techniques, organization of work, subsequent fermentation, future use and organization of feeding (*Johnson et al., 2003; Stojanović et al., 2010*).

The aim of modern technologies of ensiling whole-plant corn is to obtain starting material with the highest nutritional value possible, to apply appropriate procedures and use some supplements that will enable better conservation and utilisation of silage (*Đorđević et al., 2011*).

The quality of whole-plant corn silage is very important in milk production on family farms. Hence, quality control is a significant part of feeding programmes (*Seglar, 2003*). Quite often, poor quality silage leads to serious metabolic disorders (*Stojkovic et al., 2012; Krnjaja et al., 2013*). Silage samples should arrive at a laboratory as soon as possible to avoid subsequent changes that can occur in quality indicators (*Solórzano et al., 2012*). Controlling silage quality with appropriate laboratory analyses is part of good agricultural practices required in the diet of all cattle categories (*Fulgueira et al., 2007*).

The aim of this study was to use chemical and microbiological analyses in order to determine the quality of whole-plant corn silage produced on family farms.

Material and Methods

The study was conducted on 18 family farms from 17 places and 9 regions of the Republic of Serbia, in the period from 1st October 2010 to 31st November 2011 (Table 1). Depending on their production (milk, milk and meat), these family farms had Simmental and/or Holstein-Friesian cattle.

Table 1. Number of farmers, cattle breeds, districts and places included in the study

Number of farmers	Number of cattle breeds	Number of districts	Number of places
18	2	9	17

In the study period, the farms had on average 1,276 heads of all categories of Simmental and Holstein-Friesian cattle, comprising 615 cows, 197 calves, 317 heifers and 147 beef cattle.

The samples of whole-plant corn silage were taken from each farm and delivered in plastic bags to accredited laboratories for chemical and microbiological testing within the Institute for Animal Husbandry in Belgrade. Standard quantitative laboratory methods (based on a gravimetric, volumetric and potentiometric titration), identified the following indicators of silage quality: total moisture, crude protein, crude fat, crude fibre and pH value. Organic acid levels were determined and a quality assessment was carried out (the Flieg score). Microbiological analysis showed the characteristics of the silage in terms of total bacterial count, total mould count, sulphate-reducing Clostridia, isolation and identification of Salmonella and type of mould and yeast. The quality of the sample was determined adhering to the provisions from the "Rulebook on the quality of animal feed" (Official Gazette of the Republic of Serbia, No. 4, 2010, Article 101 and 102).

The experimental results were statistically analysed using STATISTICA v6. StatSoft, Inc. (2003).

Results and Discussion

Quality control of the whole-plant corn silage on family farms is necessary for optimizing the diet of all cattle categories on a farm.

Table 2. Chemical composition and pH value of whole-plant corn silage

Farm	Total moisture (%)	Crude protein (%)	Crude fat (%)	Crude cellulose (%)	pH
1	62.94	2.49	1.12	9.05	3.88
2	63.01	2.39	1.21	7.91	4.09
3	64.56	2.00	0.87	9.19	4.09
4	68.04	1.73	0.90	6.81	4.16
5	65.50	1.08	1.39	8.33	3.78
6	71.41	1.17	1.17	5.72	3.95
7	63.18	1.96	1.23	8.66	4.06
8	59.11	3.06	1.50	9.98	4.05
9	72.01	1.07	0.72	7.63	4.08
10	64.21	2.05	1.01	8.56	4.08
11	65.63	2.13	1.11	8.97	3.91
12	70.21	1.46	0.90	8.72	3.88
13	71.20	2.44	1.38	7.76	3.74
14	70.57	2.14	1.84	7.40	3.50
15	68.53	2.13	1.67	8.14	3.73
16	66.41	2.62	1.74	8.38	3.80
17	69.43	1.81	1.26	6.95	3.58
18	58.79	2.65	1.66	7.52	3.93
Average	66.37	2.07	1.26	8.09	3.90
SD	4.08	0.56	0.33	1.01	0.19
CV	6.14	27.14	25.85	12.46	4.81
Min	58.79	1.07	0.72	5.72	3.50
Max	72.01	3.06	1.84	9.98	4.16

The average moisture in the analysed samples was 66.37% (58.79-72.01%), while the dry matter was on average 33.63% (27.99-41.21%) (Table 2). The variation in dry matter can result in changes in levels of nutrients and energy. Adequate silage moisture is crucially important for the level of compression and the quality of fermentation (*Seglar, 2003*). Most recommendations indicate that the optimal moisture should be 65-70%, and dry matter content 30-35%. Crude protein averaged 2.07 (1.07-3.06%), crude fat 1.26% (0.72-1.84%) and crude cellulose 8.09 (5.72-9.98%). The average pH value taken as an indicator of fermentation quality was 3.90 (3.50-4.16). *Kung and Shaver (2001)* reported that the optimum pH value of corn silage ranged 3.7-4.2.

Table 3. Volatile fatty acids in whole-plant corn silage

Farm	Lactic acid (%)	Acetic acid (%)	Butyric acid (%)
1	2.10	0.98	0
2	1.35	0.64	0
3	1.59	0.78	0.08
4	0.81	0.47	0
5	2.36	0.39	0
6	2.32	1.52	0.01
7	1.63	0.34	0
8	2.10	0.24	0.27
9	0.67	1.33	0
10	2.47	1.18	0
11	1.64	0.89	0
12	1.83	0.50	0
13	2.27	0.78	0
14	2.47	0.49	0
15	2.07	0.47	0
16	2.32	0.58	0
17	2.01	0.37	0
18	1.75	0.20	0
Average	1.87	0.68	0.02
Min	0.67	0.20	0
Max	2.47	1.52	0.27

Chemical analysis showed the level of volatile fatty acids in the silage. Lactic acid was 1.87% (0.67-2.47%), acetic acid 0.68% (0.20- 1.52%) and butyric acid 0.02% (0-0.27%) (Table 3). The results show that the main product of sugar fermentation in the silage is lactic acid, which exhibits pronounced bactericidal but poor fungicidal effect. The silage also contained acetic and a less amount of butyric acid. In addition to propionic acid, they have very fungicidal properties so only fewer amounts of these acids are acceptable (*Đorđević et al., 2011*).

Table 4. Proportion of volatile fatty acids (Flieg score) in whole-plant corn silage

Farm	Lactic acid (%)	Acetic acid (%)	Butyric acid (%)	Flieg score
1	68.16	31.84	0	Very good
2	67.94	32.06	0	Very good
3	64.91	31.94	3.15	Good
4	63.40	36.60	0	Good
5	85.77	14.23	0	Very good
6	60.31	39.50	0.19	Good
7	82.55	17.45	0	Very good
8	80.49	9.35	10.16	Satisfactory
9	33.33	66.67	0	Satisfactory
10	67.54	32.46	0	Very good
11	64.78	35.22	0	Good
12	78.34	21.66	0	Very good
13	74.50	25.50	0	Very good
14	83.57	16.43	0	Very good
15	81.64	18.36	0	Very good
16	79.91	20.09	0	Very good
17	84.43	15.57	0	Very good
18	88.81	11.19	0	Very good
Average	72.80	26.45	0.75	Very good
Min	33.33	9.35	0	Satisfactory
Max	88.81	66.67	10.16	Very good

An important indicator of optimum fermentation is the level and proportion of lactic, acetic and butyric acid (Table 4). Increased production of lactic acid reduces pH value, which prevents the growth of undesirable microorganisms. Good whole-plant corn silage should contain minimum 65 to 70% total acid (*Kung and Shaver, 2001*). The average proportion of lactic acid in the analysed silage was 72.80% (33.33-88.81%), acetic acid 26.45% (9.35-66.67%) and butyric acid 0.75% (0-10.16%). Quality rating was conducting by using the Flieg score. The samples from 12 farms was graded as very good, while the silage from the other farms was evaluated as good (4 farms) and satisfactory (2 farms).

Table 5. Microbiological analysis of whole-plant corn silage

Farm	Total bacterial count (in 1 g)	Total Mould count (in 1 g)	Sulphate-reducing Clostridia (in 50 g)	Isolation and identification of Salmonella (in 50 g)
1	80,000	Not isolated	Not isolated	Not isolated
2	90,000	20,000	Not isolated	Not isolated
3	100,000	80,000	Not isolated	Not isolated
4	80,000	Not isolated	Not isolated	Not isolated
5	10,000	Not isolated	Not isolated	Not isolated
6	20,000	Not isolated	Not isolated	Not isolated
7	20,000	Not isolated	Not isolated	Not isolated
8	60,000	5,000	Not isolated	Not isolated
9	10,000	10,000	Not isolated	Not isolated
10	30,000	5,000	Not isolated	Not isolated
11	3,000,000	1,000	Not isolated	Not isolated
12	60,000	Not isolated	Not isolated	Not isolated
13	200,000	1,000	Not isolated	Not isolated
14	20,000	1,000	Not isolated	Not isolated
15	1,900,000	Not isolated	Not isolated	Not isolated
16	1,400,000	2,000	Not isolated	Not isolated
17	20,000	1,000	Not isolated	Not isolated
18	200,000	Not isolated	Not isolated	Not isolated

The analysis of microbiological quality of the samples showed, on average, 405,556 bacteria (10,000-3,000,000), compared to 12,000,000, what is the allowed bacterial count that can be found in silage (Table 5). The sample contained on average 7,000 moulds (0-80,000), compared to the allowed 200,000. Sulphate-reducing Clostridia were not identified. Neither were Salmonella.

Table 6. Determination of microorganisms (mould, yeasts) and sample quality

Farm	These analyses are determined		Sample quality
	Moulds	Yeasts	
1	Not determined	Saccharomyces cerevisiae	Corresponds
2	Mucor	Not determined	Corresponds
3	Mucor, Aspergillus flavus, Penicilium	Not determined	Corresponds
4	Not determined	Saccharomyces cerevisiae	Corresponds
5	Not determined	Not determined	Corresponds
6	Not determined	Not determined	Corresponds
7	Not determined	Saccharomyces cerevisiae	Corresponds
8	Penicilium	Saccharomyces cerevisiae	Corresponds
9	Mucor, Penicilium	Not determined	Corresponds
10	Penicilium	Not determined	Corresponds
11	Mucor, Rhisopus, Aspergillus flavus, A. ochraceus, A.fumigatus, A.niger, Penicilium, Fusarium, Alternaria	Not determined	Corresponds
12	Not determined	Not determined	Corresponds
13	Mucor, Aspergillus flavus	Not determined	Corresponds
14	Penicilium	Not determined	Corresponds
15	Not determined	Saccharomyces cerevisiae	Corresponds
16	Mucor	Not determined	Corresponds
17	Fusarium	Saccharomyces cerevisiae	Corresponds
18	Not determined	Saccharomyces cerevisiae	Corresponds

One should expect quality whole-plant corn silage should contain high levels of nutrients and energy, and be microbiologically stable over a long storage period. The study showed the presence of certain types of microorganisms (mould, yeasts) in the silage from 10 farms, and the presence of yeast on the silage from 7 farms (Table 6). The samples were entirely in line with the provisions in the "Rulebook on the quality of animal feed" (Official Gazette of the Republic of Serbia, No. 4, 2010).

Conclusions

The analysed samples of whole-plant corn silage contained on average 66.37% moisture (58.79-72.01%), 33.63% dry matter (27.99-41.21%), 2.07% crude protein (1.07-3.06%), 1.26% crude fat (0.72-1.84%) and 8.09% crude cellulose (5.72-9.98%). The average pH value, taken as an indicator of fermentation quality was 3.90 (3.50-4.16). When it comes to volatile fatty acids, lactic acid was 1.87% (0.67-2.47%), acetic acid 0.68% (12.20 to 1.52%) and

butyric acid 0.02% (0-0.27%). The average proportion of lactic acid in the silage was 72.80% (33.33-88.81%), acetic acid 26.45% (9.35-66.67%) and butyric acid 0.75% (0-10.16%). Quality rating of the silage was conducted by using the Flieg score. The samples from 12 farms were graded as very good, while the silage from the other farms was evaluated as good (4 farms) and satisfactory (2 farms). The analysis of microbiological quality showed that the silage on average contained 405,556 bacteria (10,000-3,000,000). The quality of the samples matched the requirements prescribed by the rulebook.

Kvalitet silaže cele biljke kukuruza na porodičnim govedarskim farmama

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Rezime

Savremene tehnologija siliranja cele biljke kukuruza imaju za cilj dobijanje kvalitetne silažne mase što veće hranljive vrednosti uz primenu postupaka koji će omogućiti maksimalno očuvanje i visok nivo iskoristivosti hranljive vrednosti. Stalno praćenje kvaliteta važan je deo programa ishrane svih kategorija goveda. Cilj ovoga istraživanja bio je utvrđivanje kvaliteta silaže cele biljke kukuruza proizvedene na porodičnim govedarskim farmama primenom hemijske i mikrobiološke analize. U analiziranim uzorcima silaže cele biljke kukuruza prosečan sadržaj vlage iznosio je 66,37 % (58,79-72,01%), sadržaj suve materije 33,63 % (27,99-41,21%), sirovog proteina 2,07% (1,07-3,06%), sirove masti 1,26% (0,72-1,84%) i sirove celuloze 8,09 (5,72-9,98%). Prosečna pH vrednost iznosila je 3,90 (3,50-4,16). Sadržaj isparljivih masnih kiselina za mlečnu kiselinu iznosio je 1,87% (0,67-2,47%), sirćetnu kiselinu 0,68% (0,20-1,52%) i buternu kiselinu 0,02% (0-0,27%). Prosečna proporcija mlečne kiseline u analiziranoj silaži iznosila je 72,80% (33,33-88,81%), sirćetne kiseline 26,45% (9,35-66,67%) i buterne kiseline 0,75% (0-10,16%). Ocena kvaliteta silaže po Flieg-u je pokazala da su uzorci sa 12 govedarskih farmi dobili ocenu vrlo dobar, dok je na ostalim farmama silaža ocenjena kao dobra (4 farme) i zadovoljavajuća (2 farme). Analizom mikrobiološkog kvaliteta utvrđeno je da se u uzorcim silaže nalazilo prosečno 405.556 (10.000-3.000.000) bakterija i 7.000 (0-80.000) plesni. Sulforedukujuće klostridije i salmonele nisu identifikovane. Uzorci su odgovarali uslovima kvaliteta koje propisuje pravilnik.

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THE QUALITY OF MAIZE PLANT SILAGES ENSILED IN POLYETHYLENE FILMS

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Abstract: The ensiling of maize plants (FAO maturity group 600) was performed in polyethylene films (in a form of tunnels) with a "Budissa Bag" silo press (Budissa Agroservice GmbH, Germany) at 4-5 bar pressure. The films were 270 cm in diameter and 65 m long. The ensiling of the plant material was carried out in "Dragan Marković" company in Obrenovac (Serbia). Six weeks after ensiling, the representative samples from eight polyethylene films were analysed. In five films, the samples contained less than 33% of dry matter (DM) (30.21-32.9) and in other three films DM was higher, amounting to 33% (36.24-39.47). Lactic acid in the silage with less than 33% of DM was high, and amounted on average to 68.22%, whereas lactic acid in the silage with more than 33% of DM was under 62.30%. Conversely, acetic acid had the opposite trend. It amounted to 26.42% and 30.90%, respectively, being higher in the silage with higher DM. Free butyric acid was not detected (ND). Bound butyric acid was 2.21% and 3.74%. The pH values of silages were similar (3.7:3.90). These silages were assessed according to the DLG-assessment system. The average amount of points in the silages with less than 33% of DM was 47 (Class I), and in the silages with more than 33 % of DM it was insignificantly lower and amounted to 45. The silages from two films were assessed as the Class I and from one film as the Class II (silage with 39.47% of DM). No losses were detected in the silages prepared in polyethylene films, so this method of the ensiling and storage of silage has the advantage from the nutritive, economic and environmental aspects over concrete trench silos (losses from 10% to 15%). This type of the silage resulted in good organoleptic and nutritive properties, so cattle were eager to consume them.

Key words: Maize plant, silage, polyethylene film

Introduction

One of the most important factors in the livestock production is feed quality and safety. When it comes to feed safety, and especially the safety of

ensiled feed, storage conditions make the essential prerequisite for preserving its quality. Ensiling is a biotechnological method of preservation of wet forage and concentrated feed, based on a series of complex and intensive chemical, biochemical and microbiological processes. The most important product of these processes is lactic acid. It is produced by the fermentation of water-soluble carbohydrates in an anaerobic environment, due to the activity of lactic bacteria. Lactic acid is a natural preservative and it has bacteriostatic and bactericidal effect, preventing spoilage of silage and losses of nutrients *(Adamović et al., 2005).* On the other hand, organic acids produced during ensiling do not have adverse effects on moulds (they can grow between pH 3 and 8), so these microorganisms and their toxic metabolites (mycotoxins) can sometimes be found in ensiled feed. During the initial stages of ensiling, after oxygen depletion, strict aerobes (*Fusarium* species) are first to disappear, being followed by other, so-called field mycobiota (*Alternaria* and *Cladosporium* spp.). Dominant mycobiota become tolerant to oxygen deficiency, most common of which are some *Mucorales* and *Penicillium* species, *Aspergillus fumigatus*, *Trichoderma viride*, *Geotrichum candidum, Paecilomyces variotii* and *Monascus ruber (Bočarov-Stančić et al., 2014)*.

For successful ensiling and preserving the quality of silage, apart from following the basic principles of ensiling (taking into account an adequate level of dry matter, plant phenophases, mass compressing, minimum sugar, material fragmentation, etc.), the most important factor is the quality of ensiling facilities (*Dinić and Đorđević, 2005; Downing et al., 2008*). The construction of silo facilities should protect silage and haylage from air inflow, thus providing quality fermentation and preventing subsequent fermentation. Moreover, these facilities have to protect feed from groundwater, accumulation and penetration of water sediments, and contamination with microorganisms from the environment *(Dinić and Đorđević, 2005).*

The aim of this study was to investigate the ensiling of maize plants in polyethylene films, and to determine the quality of the produced silage.

Material and Methods

The ensiling of maize plants (FAO maturity group 600) was carried out at “Dragan Marković” company in Obrenovac (Serbia). Harvesting and chopping of the maize plants were carried out with a "Krone Big X 500" combine (Maschinenfabrik Bernard Krone GmbH**,** Germany), in the first half of September 2014. The ensiling of the maize plants was performed in polyethylene films (in a form of tunnels) with a "Budissa Bag" silo press (Budissa Agroservice GmbH, Germany) at 4-5 bar pressure. The films in which the biomass was filled under

pressure had a diameter of 270 cm. They were 65 m long and 0.28 mm thick, with the capacity of 250 t of ensiled mass. The maize plant cut-outs were 0.7-1.0 cm long. In five films, DM was less than 33% while in the remaining three films it was over 33%. After filling, the films were closed by folding the ends and covering them with soil, thus protecting the ensiled material from the inflow of air. The films were then perforated on the upper side, making a 3 cm hole in order to discharge residual air. After three days, the hole was closed by using a part of the film as a hatch. Bacterial inoculants were not added in the silages.

Figure 1 and 2. Press for filling polyethylene films with fresh biomass

The chemical composition and quality of the silages were determined six weeks after ensiling. The analysis of the chemical composition of the silages and the evaluation of their quality were conducted by "EKO-LAB" laboratory in Belgrade (Serbia). The chemical composition (dry matter, crude ash, crude fat, crude fibre, crude protein, nitrogen free extract) and pH of the silages was determined according the standard chemical method. Organic acids (lactic, acetic and butyric) in the silages were determined with the methods of destillation and titration and and expressed in percentage (%), according to total acids. Bound acids

(acetic and butyric) were determined from the difference between total and free acids. The class of silage was determined according to the DLG assessment system *(Đorđević and Dinić, 2007).*

Results and discussion

In five films, dry matter (DM) was less than 33% (30.21-32.9%) while in the remaining three films it was over 33% (36.24-39.47%). The level of crude ash, crude fibre, crude protein and NFE was slightly higher in silages with higher DM (Table 1).

Table 1. Chemical composition and quality of maize plant silages

Parameter	**<33% DM**	**>33% DM**
Dry matter, variation (%)	**30.21-32.90**	**36.24-39.47**
Dry matter, average (%)	31.27	38.18
Crude ash (%)	1.23	1.60
Crude fat (%)	0.66	0.65
Crude fibre (%)	7.07	7.81
Crude protein (%)	2.12	2.64
NFE (%)	20.19	25.48

NFE - nitrogen free extract

Lactic acid was high in the silage with less than 33% of DM. Its average value was 68.22%. The silage with more than 33% of DM had lower lactic acid and its average value was 62.30% (Table 2). Conversely, the level of acetic acid had the opposite trend; it increased from 26.42% to 30.90%, being higher in the silage with higher DM. In the tested samples, free butyric acid was not found, unlike bound butyric acid that amounted on average to 2.21% and 3.74%, respectively. The pH values of all the investigated silages were in optimal limits, from 3.9 to 3.9. The number of points per DLG-assessment in the silage with less than 33% of DM was 47, and in the silage with more than 33% DM it was slightly lower and amounted to 45 points. All five silages with less than DM 33% were assessed as the first class silage. Two silages with more than 33% of DM were assessed as the first class and one silage (39.47% DM) as the second class. No losses were detected in the silages prepared in polyethylene films, so this method of ensiling and storage of silage, has the advantage from the nutritive, economic and environmental aspect over concrete trench silos (losses from 10% to 15%). The both methods of ensiling resulted in silage with good organoleptic and nutritive properties that cattle were eager to consume.

Table 2. Level of acids and pH, and the class of silage

Parameter	**<33 %DM**	**>33% DM**
Total lactic acid (%)	**68.22**	**62.30**
Free acetic acid (%)	26.42	30.90
Bound acetic acid, %	3.15	3.06
Free butyric acid (%)	ND	ND
Bound butyric acid (%)	2.21	3.74
pH	3,70	3.91
Points	47	45
Sample class I	5	2
Sample class II	0	1

ND - Not detected

After ensiling maize plants with higher DM (50%) in similar films, *Zimmer et al. (2009)* determined that silage from one film had a similar level of lactic acid (62.26%) and acetic acid (37.74%) at pH 4.17. In the silage from the other film (with 43% DM), they found a significantly lower levels of lactic acid (11.49%), while acetic acid was dominant (88.51%), with pH 4.02. These results indicate that maybe there have been an oversight in the ensiling process in the second film. The reason for such a ratio between lactic and acetic acid may be higher quantities of air remained in the second film, which favoured the growth of acetic acid, producing bacteria and tremendous amounts of acetic acid. It should be noted that these authors did not detected the presence of butyric acid in either of the two analysed silages.

Adamović et al. (2014) ensiled (*Helianthus tuberosus L*) in a polyethylene film of the same performance, using the same type of press and recorded 51.07% and 57.01% of lactic acid, as well as an increased level of acetic acid (42.99% and 48.93%). The reason for such an increase in the total amount of acetic acid probably lies in the specificity of the ensiling process in a film. In this case, the compaction of the silage mass in the film was probably of lower intensity, thus creating good conditions for the activity of lactic bacteria and therefore higher production of that acid. Similar conclusions about the importance of mass compaction and elimination of air from silage were reported by *Đorđevic et al. (2012 and 2015)* and *Adamović et al. (2015).* Other authors (*Wagner et al.,* 2004; *Bhandari et al., 2008*; *McEniry et al., 2010; Yildiz et al., 2010)* also stated that dry matter and crude fibre in silage mass, together with the length of the cut-outs, could significantly affect the quality and intensity of biomass compaction.

Conclusion

The ensiling of maize plants (<33% of DM) in polyethylene films resulted in high quality silages. The dominant was the Class I, with optimal values of lactic acid and pH. The quality of maize plant silages with higher DM (>33%) was also satisfactory; Two films contained the Class I silage. The silage with the highest DM (39.47%) was assessed as the Class II silage. The process of ensiling is fast and it does not require mechanization for compaction. Moreover, the chance of contaminating silage with external microorganisms is minimized, as well as the chance of running out of juices and losing of nutrients. No losses from damage were detected in the silages of prepared in polyethylene films. Films can be placed in the most suitable places in the yard or in the field. They greatly contribute to environmental protection, and therefore this method may be given a preference over the other methods of storage of silage.

Kvalitet silaže biljke kukuruza silirane u polietilenskoj foliji

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Rezime

Siliranje biljke kukuruza (FAO grupa 600) je obavljeno u polietilenskim folijama (oblik tunela) uz pomoć prese tipa "Budissa Bag" (Budissa Agroservice GmbH, Germany) uz pritisak od 4-5 bara. Prečnik folije je iznosio 270 cm a dužina 65 m. Siliranje je obavljeno u preduzeću AD "Dragan Marković" u Obrenovcu, Srbija. Šest nedelja posle siliranja analizirani su reprezentativni uzorci iz osam polietilenskih folija. U pet folija prosečan sadržaj suve materije (SM) bio je <33% (30.21-32.9%) a kod preostale tri folije >33% (36.24-39.47%). Sadržaj mlečne kiseline u folijama sa <33% (SM) bio je visok i u proseku je iznosio 68.22% dok je u folijama sa >33% SM bio niži i iznosio je 62.30%. Nasuprot tome sadržaj sirćetne kiseline imao je obrnut trend i iznosio je 26.42:30.90%, odnosno bio je viši u silaži sa većim sadržajem SM. Slobodna buterna kiselina nije dokazana ni u jednoj od analiziranih silaža, dok je vezana buterna kiselina u proseku iznosila 2.21:3.74%. Vrednost pH silaže kretala se u okviru optimalnih vrednosti. Broj poena izračunat po DLG sistemu ocene u silaži sa <33% SM iznosio je 47, a u silaži >33% SM je bio neznatno manji i iznosio 45 poena. Svih pet silaža sa sadržajem SM <33% svrstane su u I klasu. Silaža sa sadržajem SM >33% u dve folije je ocenjena I klasom, dok je jedna silaža (sadržaj SM od 39.47%) ocenjena II klasom. Gubici u silaži u polietilenskoj foliji, usled kvarenja, nisu primećeni. Iz

tog razloga ovaj način siliranja u poređenju sa siliranjem u trenč silosima u kojima gubici mogu da budu i 10% do 15%, ima prednosti sa nutritivne i ekonomske tačke gledišta, kao i sa aspekta zaštite životne sredine. Sve silaže su imale dobre organoleptičke osobine (izgled, boja, miris) i životinje su ih rado konzumirale.

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MOULDS AND MYCOTOXINS IN MAIZE AND GRASS MIXTURES WITH LEGUME SILAGE

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Abstract: Maize and grass mixtures with legume silage are important feedstuffs for intensive livestock production. Mould fungi growth and mycotoxins production can reduce nutritional quality of feed and cause animal diseases called mycotoxicosis. Therefore, in this study, a survey of moulds and natural occurrence mycotoxins in maize and grass mixtures with legume silage were determined. A total of 35 different silage samples (maize grain silage (n=15), whole maize plant silage (n=8), grass mixture with white clover silage (n=6) and grass mixture with bird's-foot-trefoil silage (n=6)) were analysed. According to the pour-plate method a total fungal count was determined, while levels of aflatoxin B_1 (AFB_1), deoxynivalenol (DON) and total fumonisins FB_1, FB_2 and FB_3 (FBs) were detected by enzyme-linked immune sorbent assay (ELISA) method.

Based on microbiological analyses from 33.33 to 62.5% tested silage samples were contaminated with fungal species. In all the tested samples total fungal count ranged from 0 to 1 x 10^4 CFU g^{-1}. Depending on types of silage three potential toxigenic fungal genera, *Aspergillus*, *Fusarium* and *Penicillium* were identified. In mycotoxicological analyses aflatoxin B_1 (AFB_1), deoxynivalenol (DON) and total fumonisins (FBs) were detected in all the tested samples (100%), with relative high level of AFB_1 in silage samples of gras mixtures with legume.

Based on obtained results it can be concluded that all types of silage were microbiologically and mycotoxicologicaly safe, except the samples of grass mixtures with legume where anallowed level of AFB_1, for the feeding of dairy cattle, were detected. These results suggest the need for continuous microbiological and mycotoxicological control of the ensiled material in order to prevent potential risks for human and animal health along the food chain.

Key words: moulds, mycotoxins, maize and grass mixtures with legume silages

Introduction

The production of silage is the best way to preserve forage over a long period of time of detrimental microorganisms, including toxigenic fungi and to maintain its nutritional quality and safety *(Shimshoni et al., 2013)*. Lactic acid bacteria (LAB) have an important role in the process of preservation of moist forages for animal feeding (silage). This process is carried out in anaerobic conditions, where the LAB reduces the pH by producing the lactic acid. These conditions are unfavourable for the growth and proliferation of spoilage microorganisms like toxigenic fungi (moulds), which are less tolerant to acidic conditions. However, poor condition during the storage period could lead to undesirable development of mould and other microorganisms, including yeasts *(Garon et al., 2006)*. Maize silages of grinding grain and of the whole plant are a widely used feedstuff for livestock production, which may be contaminated with mycotoxins, pre- and post-harvest *(Drejer Storm et al., 2014)*. Likewise, the conservation of legume represents the need to store excess of pastures – alfalfa, winter soilage, etc. *(Fulgueira et al., 2007)*. The contamination of silage with the moulds and their secondary metabolites, mycotoxins, represents a serious hazard to human and animal health. The most commonly toxigenic fungal species, belong to *Aspergillus flavus*, *Fusarium verticillioides* and *F. proliferatum*, which are able to grow on maize and other grasses byproducing mycotoxins at the preharvest stage *(Miller, 2001)*. Among the other mould species, *Alternaria* spp., *Aspergillus fumigatus*, *Byssochlamys* spp., *Monascus* spp., *Mucor* spp., *Penicillium* spp., and *Trichoderma* spp. etc. are recognized as the most frequently contaminants in ensilaged feed *(Cheli et al., 2013; Shimshoni et al., 2013)*.

Ensiled material is often produced from the fresh plant material, which can be infected with toxigenic fungi and their mycotoxins still in the field. This adversely affects the ensiling process and reduces the nutritional value of the silage. The presence of toxin-producing fungi is particularly increases after opening the silo when the oxygen facilitate more rapid development (increase) of mould causing the deterioration of the quality of the silage material *(Potkański et al., 2010)*. The occurrence of moulds and mycotoxins is also favoured by climatic conditions. The high temperatures, low rainfall and drought are the suitable weather conditions for aflatoxin occurrence, while the production of mycotoxins by *Fusarium* spp. is favourised by the perfect cold weather and extremely wet growing seasons. Despite the progress that has been made in the creation of varieties resistant to the economically important pathogenic and toxigenic fungi

and the application of improved agronomic measures, harmful concentrations of mycotoxins may occur as a result of annual weather fluctuations. It is therefore considered that the impact of climate change on the occurrence of fungi and mycotoxins are more important than the influence of methods of preparation of silage *(Cheli et al., 2013).*

The most important mycotoxins in maize silage are deoxynivalenol, produced by *Fusarium graminearum*, fumonisins, produced by majority of *F. verticillioides* and *F. proliferatum* as well as aflatoxins, produced by *Aspergillus flavus* and *A. parasiticus (Streit et al., 2012).* Aflatoxin B_1 (AFB_1) is one of the important hepatotoxic and carcinogenic toxins known and ingestion of AFB_1 by cows can lead to potentially harmful aflatoxin M_1 residues in milk *(Kensler et al., 2011).* Fumonisins from B serie (FBs) have been associated with the occurrence of leukoencephalomalacia in horses and porcine pulmonary edema in pigs *(Bryden et al., 2012).* Deoxynivalenol (DON) belongs to the type B trichothecenes and it less toxic than type A trichothecenes (diacetoxyscirpenol and T-2 toxin) *(Bennett and Klich, 2003).* Consumption of mycotoxin contaminated diet may cause acute and chronic toxicity in humans and animals. Among farm animals, swine are considered to be the most sensitive species to mycotoxins *(Biagi, 2009).*

In Serbia, there are not enough data on micotoxigenic safety of different types of silage, with the exception of the data on its microbiological quality. For this reason, and due to the hazard effects that toxigenic fungi and their mycotoxins can cause to animal and human health, in this study total fungal count, fungal contamination and levels of aflatoxin B_1 (AFB_1), deoxynivalenol (DON) and total fumonisins FB_1, FB_2 and FB_3 (FBs) in maize and grass mixtures with legume silage samples were investigated.

Materials and Methods

The silage made of grass mixtures with legumes were prepared by using the plants of the second mowing (end of June) and from the second year (in 2014) after the crops seeding on experimental plots of the Institute for Animal Husbandry in Belgrade, Serbia. The grass mixture has been formed from the three grass species: *Festuca pratensis, Lolium perenne* and *Dactylis glomerata*. The % of contribution of every plant in grass mixture with white clover was 30:25:25:25 of the *Festuca pratensis, Lolium perenne, Dactylis glomerata* and white clover, respectively while their % in grass mixture with bird's-foot-trefoil was 20:30:20:30 of the *Festuca pratensis, Lolium perenne, Dactylis glomerata* and bird's-foot-trefoil, respectively.

Preparation of silage consist of whole maize plant and maize grains were conducted in late August and the end of September of 2013 and 2014, respectively.

The plant material inoculated with lactic acid bacteria and enzyme was placed in plastic bags and sealed by using a vacuum packaging machine and then stored for 90 days at room temperature.

For microbiological and mycotoxicological analysis a total of 35 silage samples (maize grain silage (n=15), whole maize plant silage (n=8), grass mixture with white clover silage (n=6) and grass mixture with bird's-foot-trefoil silage (n=6) were taken after 90 days of opening of the plastic bags. For microbiological analysis fresh samples of silage were used, whereas for the mycotoxicological analysis the samples were prepared by drying at 60°C within three days, and then pulverized in a mill (IKA A11, Germany). The total moisture content was determined by drying 3-5 g of sample in the oven at 105ºC *(SRPS ISO 6496, 2001)*.

Microbiological analysis were done according to the method *ISO 21527-1 (2008)*. For the determination of the total fungal count (CFU g^{-1}), 20 g of a tested sample was suspended in 180 ml of sterile physiological saline (0.85% NaCl) and homogenized for 2 minutes on an orbital shaker (GFL 3015, Germany). A serial dilution (from 10^{-1} to 10^{-4}) was prepared by dissolving of 1 ml of the homogenate in 9 ml of sterile saline to give a dilution of 10^{-2} and then the following dilution. According to pour-plate method, 1 ml of dilution 10^{-3} and 10^{-4} were placed in Petri dishes (Ø100), covered by Dichloran Rose Bengal agar and stored in an incubator (Memmert, Germany) for 5 days at 25°C.

Based on morphological characteristics as well as macroscopic (appearance of colonies) and microscopic (appearence of spore), the fungi were identified according to Watanabe (1994).

The presence of AFB_1, DON and FBs were determined using the competitive ELISA method according to the manufacturer's instructions Celer Tecna® ELISA kits. The limit of detection for AFB_1, DON and FBs were 1 μg kg^{-1}, 40 μg kg^{-1} and 750 μg kg^{-1}, respectively.

For statistacal analyses of data GLM procedure of SPSS (IBM, Statistic 20) software was used. If the effect of main factor was found significant an LSD test was used to evaluate the significant of difference. The incidence of potential fungal species (%) was calculated as number of samples with fungal species x 100/total number of samples.

Results and Discussion

The average moisture content in the analyzed samples was higher in the samples of silage grass mixtures with legume, with white clover (75.33%) and with bird's-foot-trefoil (75.02%) compared to the silage samples of grain maize (41.14%) and the whole maize plant (49.39 %).

Microbiological testing revealed that 33.33 to 62.5% of the silage samples were contaminated with potentially toxigenic fungi. Whole maize plant silage samples were mostly contaminated with fungi (62.5%), followed by maize grain and grass mixture with white clover silage samples (50%) wheras at least contaminated silage samples were grass mixture with bird's-foot-trefoil (33.33%). Total fungal count ranged from 0 to 1 x 10^4 cfu g^{-1}. Among the potentially toxigenic fungal species, *Aspergillus* spp., *Fusarium* spp. and *Penicillium* spp. were identified in grass mixture with white clover silage samples, followed by *Fusarium* spp. and *Penicillium* spp. in maize grain and maize whole plant silage samples and *Aspergillus* spp. and *Fusarium* spp. in grass mixture with bird's-foot-trefoil silage samples (Table 1).

Table 1. Total fungal colony count of *Aspergillus, Fusarium* and *Penicillium* species (CFU g^{-1}) in maize and grass mixtures with legume silage samples and percentage of mould-contaminated samples

Types of Silage	Total fungal count (CFU g^{-1})			Mould - contaminated samples (%)
	Aspergillus spp.	*Fusarium* spp.	*Penicillium* spp.	
Maize grain	0	0 – 1 x 10^4	0 – 1 x 10^4	50
Whole maize plant	0	0 – 7 x 10^3	0 – 1 x 10^2	62.5
Grass mixture with white clover	0 – 1 x 10^4	0 – 1 x 10^4	0 – 3 x 10^3	50
Grass mixture with bird's-foot-trefoil	0 – 5 x 10^3	0 – 3 x 10^3	0	33.33

According to the recommendations of the Good Manufacturing Practises International *(GMP, 2008)* the tested silage samples were not exceed the permitted total fungal count in animal feed (1 x 10^4 CFU g-1). In Serbia, according to previous studies of *Krnjaja et al. (2012, 2013*) up to 10 x 10^3 CFU g^{-1}, 1 x 10^3 CFU g^{-1} and 2 x 10^3 CFU g^{-1} was showed in the samples of silage maize grain, whole plant maize silage, and alfalfa and grass silage, respectively. In the silage samples of maize grains the fungal species from five genera *Aspergillus*, *Fusarium*, *Mucor*, *Penicillium* and *Rhizopus* were identified *(Krnjaja et al., 2012)*. Similar to the above mentioned results, in Argentina, *Gonzalez-Pereyra et al. (2011)* established that during the three studied year (2006-2008) 14 to 68% of the samples of silage from whole maize plants were contaminated (with the total fungal count from 0 to 10^8 CFU g^{-1}) with *Aspergillus, Fusarium* and *Penicillium*, as the most common toxigenic species.

Based on mycotoxicological analyses it was obtained 100% positive samples of all the tested silage samples for the presence of AFB_1, DON and FBs.

Considering the mean values, the level of AFB_1 was significantly higher ($P \leq 0.05$) in the samples of grass mixture with white clover silage than in the other samples whereas in the samples from maize grain and whole plant maize, there were no statistically significant differences in the level of AFB_1.

DON level was significantly higher ($P \leq 0.05$) for the samples of grass mixtures with white clover and bird's-foot-trefoil silages compared to the other samples that were not demonstrated statistically significant difference between each other, in the level of DON. FBs level was significantly higher ($P \leq 0.05$) in the silage of maize grain compared to the other samples that were not demonstrated statistically differences between each other in the level of FBs. Mean DON and FB_S levels in the tested samples of different types of silage did not exceed the maximum permitted limit prescribed by the regulations of the *Europen Commission (2006)*. However, for the feeding of dairy cattle, tested silage samples of grass mixtures with legume (grass mixtures with white clover and grass mixtures with bird's-foot-trefoil) exceed the maximum permitted limit (5 μg kg-1) for AFB_1, prescribed by the regulations of the *European Commission (2003)*.

Table 2. The mean levels of aflatoxin B_1 (AFB_1), deoxynivalenol (DON) and total fumonisins (FBs) in maize and grass mixtures with legume silage samples and incidence of mycotoxin positive samples

Types of Silage	Mean level of mycotoxin (μg kg^{-1})			Incidence of mycotoxin positive samples (%)		
	AFB_1	DON	FBs	AFB_1	DON	FBs
Maize grain	1.76^{c}	282.73^{a}	3894.67^{a}	100	100	100
Whole maize plant	1.44^{c}	381.63^{a}	1527.13^{b}	100	100	100
Grass mixture with white clover	11.7^{a}	106.17^{b}	1443.33^{b}	100	100	100
Grass mixture with bird's-foot-trefoil	7.5^{b}	71.50^{b}	1886.67^{b}	100	100	100

Means followed by the different letter within a column are significantly different by LSD test at $P<0.05$ level

Similar results, but in the samples of different types of silage collected from the silo-trenches and bales were reported by *Baliukonienė et al. (2012)* in Lithuania. According to these authors, the total fungal count was higher in the silage maize samples from the silo trench. From bales the maximum total fungal count was isolated from the samples of a grass mixture silage. The most frequently isolated fungi were belong to *Aspergillus*, *Penicillium*, *Rhizomucor* and *Rhizopus*. In addition, the presence of AFB_1 and DON were detected in all the tested types of silage with the highest mean concentrations in the samples of maize silage from silo trenches (14 μg kg^{-1} and 471 μg kg^{-1}, respectively) and in the samples from

ryegrass silage bales (21.2 μg kg^{-1} and 435 μg kg^{-1}, respectively). In clover silage bales, AFB_1 and DON were detected with an mean concentration of 16.8 μg kg^{-1} and 465.4 μg kg^{-1}, respectively. In Lithuania, the occurrence and development of the fungi is caused by favourable weather conditions, therefore the feed is often contaminated with mycotoxins *(Baliukonienė et al., 2012)*. According to the published data in Serbia, AFB_1, DON and FBs were detected in the samples of maize grain silage, with the highest mean concentrations of 2.81 μg kg^{-1}, 97 μg kg^{-1} and 1902 μg kg^{-1}, respectively *(Krnjaja et al., 2012)* while in the samples of silage of whole maize plant and a grass mixtures and alfalfa DON was detected with an mean concentration of 362.6 μg kg^{-1} and 164 μg kg^{-1}, respectively *(Krnjaja et al., 2013)*.

Contrary to our results, in the Netherlands, according to the data of *Driehuis et al. (2015)* in the three-year studies (2002-2004) in the samples of silage of maize plant, an mean concentrations of DON in positive samples were 854 μg kg^{-1}, ranging from 453 μg kg^{-1} in 2003 to 1009 μg kg^{-1} in 2004. The presence of FBs was only detected in the two samples of maize silage in 2004, with FB_1 concentrations ranging from 1600 to 26,200 μg kg^{-1} and FB_2 concentration of 7800 μg kg^{-1}. In the samples of the grass silage DON and FBs were not detected. Also, in all the analysed samples of maize and grass silage AFB_1 were not detected. *Driehuis et al. (2015)* explained the increased concentration of DON in the maize silage by the favourable climatic conditions in temperate climate region. They specifically indicated that the presence of heavy rainfall between July and September in 2004, was favourable to the occurrence and intensive development of *Fusarium graminearum* on maize. Low content of FBs was conditioned by unfavourable environmental conditions of maize growth in the Netherlands, for infection by *F. verticillioides*. For AFB_1, the result was not unexpected, because the occurrence of mycotoxins is strongly related to the geographical regions with tropical and subtropical climates *(Driehuis et al., 2015)*.

In this study AFB_1 content in the samples of silage of the grass mixtures with legumes was sufficiently high compared to the samples of maize silage. This was probably due to higher moisture content (> 70%) recorded in these samples which probably caused the occurrence of intensive development of *Aspergillus* species. Also, *Aspergillus* species were not identified in the samples of maize silage, while in the samples of silage of grass mixtures their total number were until 1 x 10^4 CFU g-1 (Table 1).

Conclusion

The fungi are an integral part of epiphytic mycoflora of crops, stored and ensiled feed, therefore the production of mycotoxins is primarily caused by the presence of the fungi.

Based on the results obtained in this study it can be concluded that in the tested types of silage a potentially toxigenic fungi from the genera *Aspergillus*, *Fusarium*, and *Penicillium* were identified, where *Fusarium* spp. were identified in all the tested types of silage. Generally, all the tested samples were 100% positive on the presence of AFB_1, DON and FBs. In the samples of silage of grass mixtures with legume it was established an unallowed concentration of AFB_1 compared to the limit prescribed for the feeding of dairy cattle.

Therefore, it is important to emphasize that in order to reduce the occurrence and harmful effects of mycotoxins, the implementation of microbiological and mycotoxicological control of ensiled plant material will provide more safety product without risk on the animal and human health.

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Plesni i mikotoksini u silaži od kukuruza i travnih smeša s leguminozom

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Rezime

U intezivnoj stočarskoj proizvodnji, različite vrste silaža od kukuruza i travnih smeša s leguminozoma su važna hrana za životinje. Međutim, razvoj plesnivih gljiva i produkcija mikotoksina mogu smanjiti kvalitet silaže i prouzrokovati bolesti životinja koje se nazivaju mikotoksikoze. Zbog toga, u ovom radu proučavana je prirodna pojava plesni i mikotoksina u silaži od kukuruza i travnih smeša s leguminozama. Ukupno je ispitivano 35 uzoraka različitih vrsta silaže, silaža od zrna kukuruza (n=15), silaža od cele biljke kukuruza (n=8), silaža

od travnih smeša s belom detelinom (n=6) i silaža od travnih smeša s žutim zvezdanom (n=6). Ukupan broj gljiva određen je primenom metode razređenja, dok su aflatoksin B_1 (AFB_1), deoksinivalenol (DON) i ukupni fumonizini FB_1, FB_2 i FB_3 (FBs) detektovani primenom imunoadsorpcione enzimske metode (ELISA).

Na osnovu mikrobioloških analiza od 33,33 do 62,5% ispitivanih uzoraka silaže bilo je kontaminirano s plesnima. U svim ispitivanim uzorcima ukupan broj gljiva bio je od 0 do 1 x 10^4 CFU g^{-1}. U zavisnosti od tipa silaže identifikovana su tri potencijalno toksigena roda gljiva, *Aspergillus*, *Fusarium* i *Penicillium*. U mikotoksikološkim analizama u 100% ispitivanih uzoraka detektovani su AFB_1, DON i FBs s relativno visokim sadržajem AFB_1 u travnim smešama s leguminozom

Na osnovu dobijenih rezultata može se zaključiti da su svi tipovi silaže bili mikrobiološki i mikotoksikološki ispravni, izuzev uzoraka silaže od travnih smeša s leguminozom gde je ustanovljen nedozvoljen nivo AFB_1 za ishranu mlečnih krava. Ovi rezultati ukazuju na potrebu stalne mikrobiološke i mikotoksikološke kontrole siliranog materijala u nameri da se spreče potencijalni rizici po zdravlje ljudi i životinja u lancu ishrane.

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MYCOTOXINS IN CATTLE DIET – BIODEGRADATION IN RUMEN, OCCURRENCE AND PREVENTION OF MYCOTOXICOSES

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Abstract: Ruminants are generally considered to be more resistant to adverse effects of toxic fungal metabolites (mycotoxins) than monogastric animals, since their rumen microbiota can, to a greater or lesser extent, metabolise or degrade mycotoxins. In the last fifteen years, zearalenone (ZEA) became the most predominant mycotoxin in silages in Serbia. It was detected in 5.56-63.6% of corn silage samples (mean concentration 12-1843 µg/kg) and 100% samples of alfalfa silage (mean concentration 730-2477.5 µg/kg). The dominant post-harvest mycotoxin in corn and alfalfa silages in Serbia was aflatoxin B1 (16.7%-37.5% contaminated samples; mean concentration 0.8-7.3 µg/kg). Beside ZEA and AFB1 that were detected in diets of dairy cattle, 92.5% of cattle feed samples were contaminated with both ZEA and deoxynivalenol (DON). The technological procedure of pelleting positively affected the microbiological properties, but only partially positively affected the myco-toxicological properties of calf feed mixtures. A mineral adsorbent, based on natural zeolite and added in a quantity of 0.5% to dairy cows and calves' feed, eliminated a high percentage of ZEA residues in cow's milk and zearalenone toxicoses in calves. Adding 0.2% of organozeolite to the green mass of corn during ensiling significantly (10 to 100%) decreased the concentration of fusariotoxins ZEA, T-2 and diacetoxyscirpenol.

Key words: mycotoxins, cattle diet, biodegradation, prevention of mycotoxicoses

Introduction

Nowadays, the main preoccupation of the feed industry is the production of safe and hygienically acceptable fodder mixtures. It depends on the quality of ingredients, applied technological procedures and the stability of the end-product during storage (*Bočarov-Stančić et al. 2009*). Animal feedstuffs can carry a wide range of microbial contaminants, such as bacteria, moulds and toxic metabolites of moulds (mycotoxins). The consumption of such contaminated feedstuffs can affect animal health. The consequences are more serious for monogastric animals than for ruminants. The impact of mycotoxins on ruminants depends on the age, breed, sex, dose level and immune status of an individual animal. Besides immediate mortality and morbidity, this impact can be manifested in lower productivity, reduced weight gain, reduced feed efficiency, organ damage, reproductive interference, etc. *(Upadhaya et al., 2010).*

Biodegradation in the rumen

Ruminants are more resistant to mycotoxin intoxication than monogastric animals, since their rumen microbiota can, to a greater or lesser extent, metabolise or degrade mycotoxins. The protozoal fraction of the rumen microbial ecosystem is considered to be more important in the biodegradation of these toxic fungal metabolites, since the bacterial fraction is practically inactive in the case of zearalenone (ZEA) and ochratoxin A (OTA) *(Mašek and Šerman, 2006).*
Aflatoxins (AFs) are very poorly bioconverted in the rumen – if their concentration ranges from 1.0 to 10.0 μg/kg, less than 10% is degraded. Many ruminal bacteria are completely inhibited by these concentrations of AFs *(Westiake et al., 1989).* The product of their biotransformation is aflatoxicol – a highly toxic hydroxylated derivative of aflatoxin B1 (AFB1) *(Jouany et al., 2009).*
The products of OTA hydrolysing in the rumen are phenylalanine and OTAα, metabolites less toxic then OTA *(Hult et al., 1976),* whereas the product of OTA esterification is, equally toxic, ochratoxin C. The protozoa that hydrolyse the peptide bond of OTA are considered to play a major role in this process of biotransformation *(Mašek and Šerman, 2006).*
In the rumen, ZEA is rapidly converted into ten times more toxic metabolite – α zearalenol (90%) and much smaller quantities of, less toxic, β zearalenol (10%). Further on, α zearalenol can be hydrogenated into zearalenol *(Kennedy et al., 1998),* a product used as an animal growth stimulator. Although ZEA has no toxic effects on the rumen bacterium *Butyrivibrio fibrisolvens* and does not alter the rumen function, it is considered that protozoa are mainly responsible for the biotransformation of this fusariotoxin.
Trichothecenes are also subject to the biotransformation in the rumen. The epoxy circle of deoxinivalenol (DON) is degraded to non-toxic de-epoxy DON *(Swanson*

et al., 1986). T-2 toxin is biotransformed to less toxic metabolites (HT-2, neosolaniol and other derivatives). The degradation of diacetoxyscirpenol in the rumen is more complicated and the toxicity of its metabolites has not been determined *(Jouany et al., 2009).* Rumen bacteria (*B. fibrisolvens, Selenomonas ruminantum* and *Anaerovibrio lipolytica*) are considered to play the main role in T-2, HT-2, DON and DAS bioconversion process, in the concentrations of 10 µg/ml *(Preluski et al., 1986; Westlake et al., 1987b).*

Occurrence of mycotoxins in cattle feed

Silages and feed mixtures

The mycotoxin of greatest concern to dairy farmers is aflatoxin B1, although there are some other mycotoxins that are frequently studied and also appear to pose a threat to ruminants. These are fusariotoxins: ZEA, type A trichothecenes (DAS and T-2 toxin) and type B trichothecene (DON).

Table 1. Mycotoxin contamination of corn and alfalfa silages in Serbia

Type of silage	Mycotoxin	Incidence (%)	Average (µg/kg)	References
Whole corn plant	AFB1	100.0	7.3	*Đorđević et al. (2003)*
Corn grain	ZEA	12.5	140.0	*Mašić et al. (2003)*
Whole corn plant	AFB1	25.0	3.0	*Adamović et al. (2005)*
	ZEA	43.8	1450.0	
	DAS	43.8	620.0	
	T-2	25.0	310.0	
Corn grain	ZEA	63.6	1640.0	
	DAS	18.2	1380.0	
	T-2	27.3	500.0	
Alfalfa	AFB1	16.7	3.0	
	ZEA	100.0	730.0	
	DAS	16.7	250.0	
Alfalfa	ZEA	100.0	1280.0	*Bočarov-Stančić et al. (2005)*
	DAS	100.0	250.0	
Corn grain	AFB1	-	0.8	*Adamović et al. (2007)*
	ZEA	-	12.0	
	DAS	-	31.0	
	T-2	-	31.0	
Corn grain	ZEA	5.56	500.0	*Škrinjar et al. (2011)*
Whole corn plant	AFB1	-	4.9	*Krnjaja et al. (2013)*
	ZEA	-	1843.0	
	DON	-	1149.0	
Alfalfa and grass	AFB1	100.0	7.3	
	ZEA	100.0	2477.5	
	DON	100.0	164.0	

In the last fifteen years, ZEA became the most predominant mycotoxin in corn and alfalfa silages in Serbia. It was detected in 5.56-63.6% of corn silage samples (grain and whole plant), with the mean concentrations ranging from 12 μg/kg to 1843 μg/kg. All the samples of alfalfa silage presented in Table 1 were contaminated with this fusariotoxin; mean concentrations of ZEA varied from 730 μg/kg to 2477.5 μg/kg. Other fusariotoxins less frequently found in Serbian silage were DAS (mean concentrations 31 μg/kg-1380 μg/kg), DON (164 μg/kg-1149 μg/kg) and T-2 toxin (31-500 μg/kg). According to *Bočarov-Stančić et al. (2014)*, a dominant post-harvest mycotoxin in corn and alfalfa silages in Serbia was aflatoxin B1. Its frequency varied from 16.7% to 37.5% (mean concentrations 0.8 μg/kg-7.3 μg/kg).

Having analysed the presence of mycotoxins in feed samples collected from all over Serbia in 2002, *Mašić et al. (2003)* noted that ZEA contaminated 12.5% of dairy cattle feed samples (mean concentration 140.0 μg/kg). AFB1 was detected in only 10.0% of the same samples with the mean concentration of 55.0 μg/kg.

After a one-year mycotoxicological investigation of dairy cattle feed, *Škrinjar et al. (2008)* reported that all samples, except for one (pelleted malt spent grains) contaminated with 50 μg/kg AFB1, contained mycotoxins in concentrations lower than maximum permitted by the Serbian regulation *(Official Gazette of SFRY, No. 15/87).* Having analysed 98 samples of dairy cattle feed, the same authors *(Škrinjar et al., 2011)* noted that ZEA was present together with the most frequently observed OTA. ZEA (250-980 μg/kg) was determined only in the samples of concentrate, corn silage, fresh corn, pelleted sugar beet pulp and pelleted malt spent grains. AFB1 (14.0-18.5 μg/kg) was identified only in one sample of pelleted sugar beet pulp and pelleted malt spent grains.

In an extensive investigation of different types of cattle diets collected from family farms from ten Serbian districts, *Krnjaja et al. (2013)* ascertained the presence of ZEA in all tested samples, in a concentration from 29.2 to 2477.5 μg/kg. The highest concentration of this fusariotoxin was detected in alfalfa and grass silage, and the lowest one in beet pulp silage. In 92.5% of the samples, a combined ZEA and DON contamination was detected. The highest mean concentration of DON was detected in concentrate samples (694.2 μg/kg), and the lowest one in red clover hay (11.0 μg/kg). Almost 100% of cattle feed samples (98.5%) were contaminated with total AFB1; the concentration of this mycotoxin varied from 1.6 to 7.9 μg/kg.

In Argentina, the fourth largest beef producer in the world, the prevalent fungal genera in cattle mixed feeds were *Aspergillus* spp. (60%) and *Fusarium* spp. (66.7%) *(Gonzales Pereyra et al., 2012).* Unlike the situation in Serbia, only fusariotoxin DON was detected in 33.3% of the tested samples (≥ 1250 μg/kg).

AFB1 was present in 46.7% of mixed feeds analysed in 2006 (4-10 µg/kg), while 50% of *A. flavus* strains were able to biosynthesized the same mycotoxin *in vitro*.
Simas et al. (2007) reported about the similar situation in Brazil. The most frequently isolated fungus in dairy cattle feed (brewers grains) was *A. flavus* (42.5%). The presence of AFB1 was detected in 33.75% of the samples with contamination levels ranging from 1 µg/kg to 3 µg/kg.
The investigation of *Driehuis et al. (2008)* conducted on dairy cows' feedstuffs in the Netherlands revealed that 38 to 54% of the samples were contaminated with DON (mean concentration 273 µg/kg); the incidence of ZEA (mean concentration 28 µg/kg) was much lower (17-37%). Aflatoxin, T-2 toxin and DAS were not detected in any of the samples. The major source of mycotoxin contamination was corn silage. *Tangni et al. (2013)* reported that in 2006 mycotoxin levels in the samples from Poland overpassed the EC regulatory limits *(2006/576/EC)* for deoxynivalenol, and in 2000-2001 it happened with mycotoxin levels for zearalenone in the maize silages from Germany.

Pelleted cattle feed

One of the contemporary techniques for reducing the presence of microorganisms and their toxic metabolites in feed is thermal processing (pelleting) *(Đorđević et al., 2007)*. The influence of pelleting on microbiological and mycotoxicological contamination of calf feed mixtures during storage was presented by *Bočarov-Stančić et al. (2009a and 2009b).* Although *Aspergillus flavus*, a potential producer of AFB1, was continuously found in the powdered calf mixture from the day of production (Day 0) until the end of storage (150 days), aflatoxin B1 was not detected at all. ZEA was found only after 90 days and 150 days of storage, in both types of calf feed. Most likely, the moisture content during storage increased from initial <12% to >17% (the minimal value necessary for mycotoxin production). After 90 days of storage, a higher concentration of ZEA was found in the powdered sample (736 µg/kg) than in the pelleted one (442 µg/kg). According to these results, the applied technological procedure of pelleting positively affected the microbiological properties, but only partially positively affected the mycotoxicological properties of the calf feed mixtures.
The pelleting procedure of the calf feed mixtures supplemented with bentonite (1.5%) also improved the microbiological safety of feed, but it is impossible to conclude whether it effected the mycotoxicological quality of the investigated mixtures, since only T-2 toxin was detected at the beginning of storage, in the same quantity in the pelleted and the powdered sample (377 µg/kg) *(Adamović et al., 2009).*

Prevention of mycotoxicoses

One of the efficient and rational ways to prevent negative effects of mycotoxins is to apply non-nutritive, inorganic mineral adsorbents, based on natural or modified zeolites of domestic origin.

In order to prevent the transition of ZEA and its derivatives into the milk of cows and to prevent mycotoxicoses in calves, *Nešić et al. (2003a, b, c)* tested the use of a preparation based on natural zeolite as a mycotoxins adsorbent. In a series of experiments, these authors *(Nešić et al., 2003a, b)* concluded that, after consumption of the diet contaminated with >2000 µg/kg ZEA, the highest percentage of the same micotoxin (2.51% of the concentration in feed), was secreted in milk of the control group of cows (feed without addition of natural zeolite). In the experimental group, fed with a contaminated diet supplemented with 0.2% of natural zeolite, the average daily concentration of ZEA was lower, amounting to 128 µg/kg or 1.33% of ZEA intake through the diet. The addition of 0.5% of natural zeolite resulted in the lowest ZEA concentration in the secreted milk (32 µg/kg or 0.34% ZEA intake through the diet) and production of the highest quantity of milk. Similar results were obtained when it comes to the prevention of harmful effects of ZEA in calves' diet (*Nešić et al., 2003b*). The highest weight gain (153.0 kg) was obtained in calves fed with a contaminated diet (>2800 µg/kg ZEA) supplemented with 0.5% of natural zeolite, while the lowest weight gain (147.2 kg) was obtained in calves fed with contaminated concentrate without natural zeolite added (control group). Ingested ZEA was excreted to a significant extent through faeces; the smallest quantity of ZEA in faeces was found in the experimental group of calves fed with concentrates supplemented with 0.5% of ZEA. The authors concluded that the addition of 0.5% of a mineral adsorbent based on natural zeolite to dairy cows and calves feed, eliminates in high percentage the occurrence of ZEA residues in cows' milk and the occurrence of zearalenone toxicoses in calves.

According to *Adamović et al. (2001, 2002)*, the addition of 0.2% of organozeolite to the green corn plant mass during ensiling significantly (10 to 100%) decreased the concentration of fusariotoxins ZEA, T-2 and DAS. The addition of 0.3% of organozeolite in cows' diet contaminated with 250 µg/kg of T-2 toxin (the source of contamination was cotton seeds imported from Greece) resulted in the binding of the complete trichothecene quantity *(Adamović et al., 2003 and 2008).*

Contrary to the investigations of *Adamović et al. (2001, 2002, 2003, 2008), Đorđević et al. (2003)* did not detect any reduction of the mycotoxin content in alfalfa silage prepared with the addition of 2 g organozeolite /kg fresh alfalfa mass. Silages prepared from fresh alfalfa had more ZEA than those prepared from wilted material. However, the positive effects of the use of natural zeolite were obteined

by *Grubić et al. (2003),* but with much higher zeolite doses. This entails that ensiling of alfalfa probably requires higher doses of organozeolite, in order to reduce activities of moulds and production of mycotoxins.

Conclusions

In the last fifteen years, zearalenone became the most predominant mycotoxin in silages in Serbia. It was detected in 5.56-63.6% of corn silage samples (mean concentration 12-1843 µg/kg) and 100% samples of alfalfa silage (mean concentration 730-2477.5 µg/kg).
The dominant post-harvest mycotoxin in corn and alfalfa silages in Serbia was aflatoxin B1 (16.7%- 37.5% contaminated samples; mean concentration 0.8 -7.3 µg/kg).
Beside ZEA and AFB1 that were detected in the dairy cattle diet, in 92.5% of cattle feed samples combined ZEA and deoxynivalenol (DON) contamination was found.
The technological procedure of pelleting positively affected the microbiological properties, but only partially positively affected the mycotoxicological properties of calf feed mixtures, with or without bentonite added (1.5%).
The addition of 0.5% of a mineral adsorbent based on natural zeolite to the feed for dairy cows and calves ZEA residues in cows' milk and the occurrence of zearalenone toxicoses in calves.
The addition of 0.2% of organozeolite to the green corn plant mass during ensiling significantly (10 to 100%) decreased the concentration of fusariotoxins.
On the other hand, any reduction of the mycotoxin content in alfalfa silage prepared with the addition of the same quantity of organozeolite to fresh alfalfa mass was not obtained. The ensiling of alfalfa probably requires higher doses of organozeolite in order to reduce activities of moulds and the production of mycotoxins.

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Mikotoksini u hrani za goveda u Srbiji – biodegradacija u rumenu, rasprostranjenost i prevencija mikotoksikoza

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Rezime

Hrana za životinje može biti nosač širokog spektra mikrobiološke kontaminacije, kao što su bakterije, plesni i toksični metaboliti plesni (mikotoksini). Konzumiranje tako zagađene hrane prouzrokuje kod životinja različite zdravstvene probleme koji su znatno ozbiljniji kod nepreživara nego kod preživara. Preživari su otporniji na štetan uticaj mikotoksina jer mikroorganizmi iz buraga mogu, u većoj ili manjoj količini, metabolisati ili degradirati neke mikotoksine. Smatra se da veći deo biokonverzije mikotoksina u buragu vrši frakcija protozoa s obzirom da je bakterijska frakcija buraga praktično neaktivna u slučaju zearalenona (ZEA) i ohratoksina A (OTA). Iako mogu razgraditi neke mikotoksine mikrobioti buraga nisu otporni na štetno delovanje mikotoksina tako da se kao posledica može pojaviti smanjena razgradnja celuloze i belančevina i smanjena proizvodnja isparljivih masnih kiselina.

Najveću brigu za uzgajivače goveda i prerađivače mleka predstavlja aflatoksin B1 (AFB1), mada postoje i drugi mikotoksini koji su često proučavani i koji takođe mogu biti od značaja za preživare. U pitanju su fuzariotoksini: ZEA, trihoteceni tipa A (DAS i T-2 toksin) i trihotecen tipa B (dezoksinivalenol – DON). Poslednjih petnaestak godina dominantan mikotoksin u silaži u Srbiji je bio ZEA. On je detektovan kod 5,56-63,6% uzoraka kukuruzne silaže (prosečna koncentracija 12-1843 µg/kg) i kod 100% uzoraka silaže od lucerke (prosečna koncentracija 730-2477,5 µg/kg). Dominantan post žetveni mikotoksin u silaži od kukuruza i lucerke u Srbiji je bio aflatoksin B1 (16,7% - 37,5% kontaminiranih uzoraka, prosečna koncentracija 0,8 -7,3 µg/kg). Osim ZEA i AFB1 koji su nađeni i u hrani za mlečne krave, u 92,5% uzoraka istog tipa stočne hrane detektovana je jednovremena pojava ZEA i DON.

Tehnološki postupak peletiranja je pozitivno uticao na mikrobiološke, ali samo delimično pozitivno na mikotoksikološka svojstva krmnih smeša za ishranu teladi sa ili bez dodatka bentonita (1,5%).
Jedan od efikasnih i racionalnih načina za sprečavanje negativnih posledica delovanja mikotoksina je primena nehranljivih neorganskih mineralnih adsorbenasa baziranih na prirodnim ili modifikovanim zeolitima. Dodatak mineralnog adsobensa na bazi prirodnog zeolita u količini od 0,5% u hranu za muzne krave i telad, eliminisao je u velikom procentu pojavu ostataka ZEA u mleku krava, kao i kod teladi pojavu zearalenon toksikoza. Dodavanje organozeolita, u količini od 0,2% u zelenu kukuruznu masu, je značajno (10 do 100%) smanjilo koncentraciju fuzariotoksina ZEA, T-2, i DAS u silaži. S druge strane, uz dodatak 2 g organozeolita po kg sveže mase lucerke nije dobijeno smanjenje količine mikotoksina u silaže od lucerke. Pri siliranju lucerke verovatno je potrebno upotrebiti veće količine organozeolita u cilju redukcije aktivnosti plesni i proizvodnje mikotoksina.

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USE OF SUNFLOWER MEAL WITH WEANED PIGLETS

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Abstract: Special attention must be paid to proper nutrition of piglets, even during breast-feeding, because the sow's milk does not have a sufficient amount of protein needed. Especially after the rejection, they must have properly balanced nutrition with sufficient amount of quality proteins. The experiment was conducted on weaned piglets. In two repetitions (I and II experiment), with a control (K) group and two experimental groups O_1 and O_2 for both experiments. In a group there were 22 piglets, a total of 132 units. The first period of feeding is from 8-16 kg of body weight of piglets (lasted for 23 days), and the second period of feeding is from 16-27 kg of body weight and lasted for 22 feeding days. The experimental group O_1 was added 3% of sunflower meal to the compound mixture, the second experimental O_2 was added 4% of sunflower meal. We used conventional sunflower meal with 33% of protein. The following parameters were researched: body weight of the weaned (kg), daily growth (g), daily consumption (kg), conversion of food per kilogram of growth (kg), mortality and the health condition of piglets.

Key words: sunflower meal, weaned pigs, production results

Introduction

Nutrition of intensive pig production would have to meet the needs of animals in all nutrients, especially in proteins.

The composition of sunflower oil meal is significant considering that this is our most important oilseed crop plant. In the production of meal, on the sunflower with us there goes 80%. Contemporary oil extraction is based on the extraction with a solution of organic solvents of fat (petroleum ether, benzene, acetone, alcohol, etc.). The content of nutrients in sunflower meal depends on the extraction mode and whether the grain was pre-shelled. For example, the extracted dehulled

grain meal contains about 44% of proteins, meal of whole grain has 28% of proteins, cellulose of 15-24%. The meal has a low amount of lysine, main limiting acid, but has two times more methionine and soy cysteine.

Meals are products obtained by extraction of the oil production of technically prepared (peeled, minced, pressed and heated) seed or germ nuclei (*Regulation on the quality of the food for animals*, table 1).

Table 1. According to the Regulation on the food quality for animals (*REGULATION…*) johnnycake and meal must fulfill the quality requirements given in the following table.

Type of johnnycake or meal	Description	Protein % lowest	Humidity % highest	Cellulose % highest	Ashes% highest
Johnnycake made of partly peeled sunflower seed	Product obtained by pressing partly peeled sunflower seed(Helianthus annus); gray to roan	35	12	20	6
Meal made of partly peeled sunflower seed – I quality	Product obtained by pressing partly peeled sunflower seed (Helianthus annus); extraction and separation	42	12	12	8
Meal made of partly peeled sunflower seed – II quality	Product obtained by pressing and extraction of partly peeled sunflower seed (Helianthus annus)	37	12	18	8
Meal made of sunflower seed with low protein share – III quality	Product obtained by extraction; pressing and separation of Ruff fraction of meal from partly peeled sunflower seed	33	12	21	8
Meal made of sunflower seed with low protein share – IV quality	Product obtained from meal with lowered protein share and peel of sunflower seed	20	12	34	-

When it comes to pig nurture, the usage of sunflower meal is limited from 5 to 10% because of high presence of cellulose, but to sows in lactation the usage of grain affects the milk production favorably and should be in the nurture from 25 to 30%.

Alternative sources of protein for animal production, meet in proceedings of a symposium National Research Council U.S. *(NRC, 1973)*, table 2.

Table 2. The chemical composition and nutritional value of sunflower meal - solvent, in grams per kilogram of feed (*NRC, 1973*) makes:

Name nutrients	DM	DE	CP	Lys	Ca	P	Na	Cl	K
Sunflower Meal	%	MJ/kg	g/kg	g/kg	g/kg	g/kg	g/kg	g/kg	g/kg
	92.2	9.34	261.8	9.3	4.4	9.2	0.4	1.1	13.8

DM - Dry matter, DE - digestible energy, CP- crude protein; lys-lysine

Sunflower meal in the pig nutrition is limited, because of the high level of fibers and the deficit in some amino acids like lysine, tryptophan and threonine (*Perez et al., 1986*). Soy meal has greater energy value than sunflower meal, bare energy value for pigs nutrition, in the sunflower meal is 64% of the value that low-protein soy meal has (6 to 9 MJ/kg DM). Overall energy value for sunflower meal is very changing, and it ranges from 3.5 to 9.0 MJ/kg DM, comparing different levels of peeling seeds (*Perez et al., 1986*).

Materials and Methods

The research was done on weaned piglets. The experiment included 132 piglets, it was done in two tries (labeled as experiment I and experiment II). After the group-conrolling system was formed, the experiment was done on experimental group O_1 where 3% of sunflower meal (33% of protein) was added, and on experimental group O_2 where 4% of sunflower meal was added. The first period of nutrition lasted for 23 days, until the average growth of over 15 kg of body weight was achieved, using the mixture of 20% raw proteins, and the second period that lasted 22 days, where the average growth of body weight achieved varied from 16-27 kg, using the mixture of 18% raw proteins. Both of the experiments lasted for 45 feeding days.

The results investigated were: daily growth, daily consumption, conversion of food for a kilogram per growth.

Results and Discussion

Table 3. The influence of sunflower meal on the results of weaned piglets

Traits	I Experimental			II Experimental		
	K	O_1	O_2	K	O_1	O_2
Body weight (kg)	24.82	25.13	25.18	25.20	25.18	25.32
Daily weight gain (g)	367	*378	370	371	377	375
Daily consumption (kg)	0.869	0.894	0.879	0.884	0.895	0.893
Converse, FC ratio (kg)	2.376	2.364	2.375	2.384	2.375	2.380

*In the daily gain, there was a statistical significance between the O_1 group compared to K

group (P <0.05) for I experiment.

Based on the table 3 we can see that the piglets who weighted over 8 kg in the beginning of the experiment, achieved the average weight of over 25 kg after the end of the first and second of 45 day nutrition period. The experimental group O_1 was added with 3% of sunflower meal in the forage mixture, and to the O_2 experimental group, the 4% of sunflower meal was added. Body weights of the piglets at the end of the first experiment for K group were 24.82 kg, O_1 – 25.13 kg and O_2 – 25.18 kg. During the second experiment the K group was found with results of 25.20 kg, O_1 – 25.18 kg and O_2 – 25.32 kg.

In the daily growth of first experiment there was a statistical significance between O_1 gropu and control (K) group of piglets (P<0,05).

Daily food consumption for the first period of feeding collectively for both whole experiments was higher with O_1 and with O_2 compared to the K group, the situation was similar in the second feeding period.

Food conversion per kg gain for both experiments compared to the control group was lower with O_1 and O_2. The mortality of piglets collectively for I and II experiment was 9.09%.

The results of the impact of sunflower meal in weaned piglets' food, on the production capability demonstrated physiological and economic viability in the amount of 3% in food.

Our research results match the research results of *Srećković et al., (1966), Zlatić (1980) and Živković (1960). Sredanović Slavica et al., (2006)* investigated the influence of sunflower and soy feed, if used as a replacement for milk given to calves and piglets. In the work of *Jovanović et al., (2000),* who have investigated the best hybrid choice for consumption of people and feeds, the protein share in those types was above 22%, and in hybrids even up to 30,75%. *Marinković et al., (2003)* had experiments that included breeding the protein share, getting the sunflower flour with higher protein digestibility, and lower sugar level since its presence causes degradation of gasses in digestive tract.

There are opposing opinions about whether sunflower meal should be used in the pig nutrition. A certain number of authors claims that sunflower meal shouldn't be used in the nutrition of pigs (*CETIOM, 2003*). Other authors recommend the usage of sunflower meal only with older pigs, and only if the meal in case is the one that has 25-33% of protein (*Blair, 2007*)**.** Only high quality sunflower meal can be used with animals with greater needs in energetic and protein requirements. The usage of sunflower meal in exchange for soy meal with piglets and growing pigs, has led to decrease of food consumption, lower growth, lower meat quality (*Lipinski et al., 2002*). These lacks of sunflower meal can be eased, but not completely, by adding of lysine and oil supplements (*Shelton et al., 2001*). The usage of sunflower meal for pig nurture depends on its profitability and the local conditions. In Turkey, for

example, sunflower meal with the addition of lysine gives similar results as soy meal (*Akdag et al., 2008*).

Conclusion

Based on the results we gor from the research about the influence of sunflower meal (33% protein) in the meal on detection of producting abbilities of weaned piglets, we concluded that it is desirable to add sunflower meal to the nutrition of weaned piglets in the amount of 3%. According to our researches we can recommend the usage of sunflower meal in the case of weaned piglets of body weight bigger than 8 kg, but with the addition of synthetic lysine. The quality of meal depends on the content of peel. Certain oil factories ennoble meal in their special devices, which are used to regulate the percentage of peel. Using this method, the share of peel can be decreased to 7%, and the share of proteins can be increased up to 48%. Ennobled meal can be used to feed even the ruminants (pigs, poultry, etc.)

Upotreba suncokretove sačme kod odbijene prasadi

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Rezime

Kod intenzivnog uzgoja svinja posebnu pažnju treba obratiti na sadržaj proteina u ishrani odlučene prasadi. Sastav uljanih sačmi suncokreta značajan je s obzirom da je to naša najvažnija uljana biljna kultura (uljarica). Sačma ekstrahovanog oljuštenog zrna sadrži oko 44% proteina, sačma iz celog zrna ima oko 28% proteina, celuloze od 15-24%. Sačma suncokreta sadrži nisku količinu lizina, glavne limitirajuće kiseline, ali ima dva puta više metionina i cisteina od soje.

Ogled je izveden na odbijenim prasadima i trajao je do njihove prosečne telesne mase oko 25 kg, u dva ponavljanja, tj. kao prvi (I) i drugi (II) ogled, sa po dve ogledne grupe u oba ponavljanja (O_1 i O_2), po grupno kontrolnom sistemu. Kontrolnoj grupi (K) nije u hrani dodavana suncokretova sačma. Prvoj glednoj O_1 dodato je 3%, a drugoj oglednoj O_2 grupi 4% suncokretove sačme (33% proteina). Rezultati uticaja suncokretove sačme u obroku odbijene prasadi, na proizvodne sposobnosti pokazali su fiziološku i ekonomsku isplativost u količini od 3% u hrani.

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PREREQUISITE PROGRAMMES AND RODENT CONTROL IN LIVESTOCK PRODUCTION

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Abstract: Mechanisms promoting the control of food safety, the environment, working conditions and wellbeing of farm animals are provided in prerequisite programmes for livestock production and other areas of agricultural production. Good agricultural practices (GAP), good manufacturing practices (GMP) and sanitation standard operating procedures (SSOP) constitute the preconditions of proper functioning of a HACCP programme. These programmes prevent food contamination by various pathogens and constitute a basis for an analysis of hazards and identification of critical control points (CCP).

One of the most important SSOP points is rodent control, i.e. the control of potential hazards caused by their activities. Rodent control companies need all to be acquainted with the rodent integrated pest management (IPM), HACCP programmes and COSSA requirements for safe use of chemicals. Rodent control operators need to have sanitary certificates to ensure product safety. Still, rodent control companies have often been found in the past to breach the provisioned standards and procedures, despite the many years of implementation of IPM programmes. Records are often incomplete.

This paper describes the significance of prerequisite programmes for food safety and the HACCP programme in technological production processes. The interaction of rodent control technology, damage and possibiliites are assessed based on the prerequisites of rodent control, mode of control and effects of poor HACCP programme implementation. The importance of rodent control is stressed and a need is assumed for adjustments according to advancing food production technologies.

Key words: Prerequisite programmes, food safety, HACCP programme, rodent control, livestock production

Introduction

The HACCP system recieved a legal framework in Serbia several years ago, but an impression has been voiced that it has not been fully comprehended and that its implementation is poor. The system had been originally developed for food technology and safety of products through identification of points in a process that are critical from the microbiological, chemical or physical aspects. Before implementing a HACCP system, adequate implementation of prerequisite programmes is crucial *(Engel, 1999)*. A sanitary certificate incorporates a preventive approach to the elimination of rodents which may cause damage in food industries. A common misapprehension of agricultrual company personnel is that rodent management is a CCP or that the HACCP can be used to manage rodents.

Rodent management is a prerequisite, while a CCP is a point in food production processes that needs to be identified and monitored throughout that technological process or major consequences may occur *(MacDonald, 1996)*. The purpose of pest control in food industries is to prevent their occurrence in facilities that contain raw material, intermediate goods and finished goods. Once their presence has been detected, application of control methods is mandatory.

One of the basic musts of modern agriculture, including livestock production, is to manufacture healty food and to meet the requirements of a farm-to-table model. Inadequate preparation and processing of raw material *(Bojkovski et. al., 2011)* may cause health problems in consumers, even lethal outcome when zoonosis is the cause of infection *(Bogdanović and Stanković, 2011)*. Safety of primary meat products encompasses a variety of conditions that need to be fulfilled. Standards in breeding conditions, health and wellbeing of animals make the basis of providing safe products.

The importance of industrial rodent control is triple:

- HACCP as a concept makes rodent control mandatory, and it is committed to the care of certified rodent control companies
- Rodent control operators must be trained and aware of the importance of HACCP in food industries and industrial rodent control.
- Erroneous implementation of a HACCP programme has consequences on rodent control.

The purpose of our investigation was to analyze the interaction of prerequisites rodent management, character of control and consequences on rodent control operators from food safety aspect in livestock production. We focused on the influencing factors in livestock facilities, their CCPs, preventive points and their effect on hygiene accreditation and HACCP programme. We determined the degree of rodent invasion and assessed population density in facilities *(Hrgović et al., 1991)* and scored them using an accepted scale.

Materials and Methods

A mainly pork-producing facility (facility A) and a cattle farm (facility B) were assessed in the present study. Both facilities have protocols for implementation of HACCP programmes.

Facility inspection was carried out on a monthly basis and records of procedures *(Engel, 1999; MacDonald, 1996)* were made by certified rodent control companies. Operations were identified by ordinal numbers.

Vectors, influential factors, CCPs and preventive points (Table 2) are shown in an adjusted table *(Hardy, 2002)*. The number of CCPs and their arrangement were determined visually during inspection.

Different quantitative methods were used to estimate the degree of invasion, density of mice populations based on different terrain characteristics of each facility. A 1-5 scale was used to assess a relative rodent population density: 1 – very low, 2 – low, 3 – slightly abundant, 4 – abundant, and 5 – highly abundant *(Hrgović et al., 1991)*. Density index and dispersion degree were estimated from the feces found. Based on the shape and size of feces we identified the rodent species and their age structure. Damage and biting activity were found on food products, packaging and other wooden articles. Traces of incisor bites were detected on electrical instalation *(Hrgović et al., 1991)*, those of 1-2 mm were identified to belong to *Mus musculus*, and those of 4 mm to *Rattus norvegicus*.

Results and Disscussion

The HACCP is not an independent programme, it is rather a part of a larger system of control procedures. It needs prerequisite programmes to make it effective. The system controls *(Snyder, 2001)* elements of hazard in food production: various contaminants, facilities, chemicals, raw materials, conditions of processing and storage, and it operates through instructions and records that are being kept and signed by heads of HACCP teams. The personnel must systematically follow procedures for ensuring food safety, and understand each step in the process.

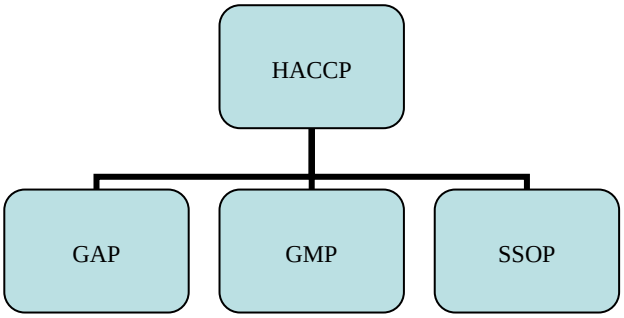

Figure 1. Interrelation of the prerequisite programmes GAP, GMP and SSOP with HACCP programme

GAP (good agricultural practices) identifies the principles of food safety during sowing, harvest and transport of fresh products, and promotes the use of general recommendations for development of good agricultural and management practices *(Zagory, 1999)*. Application of these principles aims to prevent contamination of fresh products with various pathogens. No legal or regulation duties are involved but manufacturers are required to take measures of precausion to prevent contamination. It is a document containing instructions for preventive measures.

GMP (good manufacturing practices) is a regulation that prescribes conditions for handling food, including major crops, equipment, manufacturing and other processes that are being controlled, and records are made of their divergence. The procedures described in GMP include simple actions *(Zagory, 1999)*, such as washing hands, wearing caps, etc., as well as a variaty of other more complex procedures for handling food in a manufacturing facility, and their adherence is mandatory.

SSOP (sanitation standard operation procedures) provides procedures for cleaning and maintainance of each working area according to plan. Rodent control is the third main component of this programme *(Snyder, 2001)*, and it has indicators. A rodent control programme is normally contracted and adheres to rules. Each month, a rodent control operator fills out a document that contains data about the data and location of control activity, number and species of rodents cought in traps, characteristics of control products applied, number of treatments, number of identified CCP, etc.

Table 1: Prerequisite procedures relating to rodent management, mode of rodent control and effects of poor implementation of HACCP on rodent control operation

Prerequisites for rodent management	**Mode of rodent management**	**Effects of HACCP on rodent control operation**
1. Responsibility for rodent control must be entrusted to a high-ranking manager	**1**. Advice about appropriate rodent prevention	**1**. Requires clear instructions about treatments and agreement in HACCP team on their adequacy and position
2.Adequate measures for preventing rodents from entering facility	**2**.Advice about monitoring procedures	**2**.Training of personnel about rodent species and their impact on food safety
3.Properly secured, laid and maintained rodent baits	**3**.Advice about different methods of eradication	**3**. Limited choice of active ingredients
4.Rodent control execution is entrusted to a reputed company	**4**.Keeping of correct and complete records	**4**. Limited access to facilities
5.Execution is contracted	**5**. Mapping of CCPs for monitoring	**5**.Regular and systematic cleaning may hamper detection, tracking and identification of rodents
6.Company management is informed before and after each visit by a rodent control team	**6**. Choice and method of laying chemical or biological baits	**6**.Possible impact of cleaning materials on control product
7. Control team keeps written records about rodent control activity and recommends control solutions in writing	**7**. Rodent control and food hygiene operators are HACCP-trained	**7**.Need for keeping records of each control product used in each specific treatment
8.Rodent sightings must be reported to a supervisor	**8**.Regular inspection of CCPs and written reports of each activity	**8**. Keeping of full and regular records in writing
9.Revised hygiene plans need to include routine supervision in facilities and parameters relating to rodents	**9**.Advice on potential threat to food products during rodent control treatment	
10.Guidelines provide information on a rodent control programme	**10**. Personnel training for rodent control and relation with HACCP	
	11. Assigning employees with tasks for resolving problems, and a HACCP support team	
Average evaluation score		
A good	A good	A good
B sufficient	B sufficient	B sufficient

Table 2. presents a document consisting of a series of procedures in a line of sequance and importance as prerequisites relating to rodent control, mode of rodent management and effects on rodent control operation that result from weak implementation of a HACCP programme. It is filled out on a monthly basis,

analyzed, records are kept of each irregularity, and adequate solutions are chosen and implemented without hesitation. A HACCP team is responsible for taking care of food production and quality control data also, and for managing a long-term programme for improvement of food quality within units. Poor results in that respect may have reverse impact on rodent control operators.

Even though HACCP protocols, sanitation certification programmes and rodent control contracts with certified companies had been signed by the two facilities in this study (Figure 2), we found that adequate measures for prevention of rodent entry into facilities were not implemented.

Information notices on rodent control were not posted at prominant places for the facility A personnel, while they were incomplete in facility B.

In facility A, documentation was kept regularly and contained clearly marked CCP. The personnel had not received training for rodent management and the HACCP system. A bromadiolone-based product was laid without protective boxes in offices, which was the responsibility of a rodent control company (Figure 2). Its average score was good (3) and it corresponded to the number of CCPs (Table 2). The highest risk to product safety was ascribed to birds, rodents and flies ($CCPA_{345678}$ and $CCPB_{67891011}$).

The HACCP team in facility B found that rodent occurrence was critical for food safety and that pest absence in storages should nevertheless remain a CCP. Rodent management has thus become a means of control and monitoring of pests in food production processes. It may lead to a confused perception that each bait station is a CCP. Despite regular inspection and adequate record keeping, rodents were observed during each revision visit, and it correlated with the number of CCP (17). Rodent activity and damage were the highest in rooms in which concentrate feed was stored. Cleaning and disinfection revision is required. Also, Longford traps should be laid in order to determine the number of animals at all points in which their traces had been detected and in which they may be expected to appear. Additional measures should be taken, including more treatments or change of the bromadiolone-based bait to which resistance evolution has occurred *(Šćepović et al., 2015)*.

Based on the initial document (Table 1) and data for the requirements of all three programmes, a rodent control analyst fills out one of the following lists (Figures 2 and 3). Their difference *(Barrett, 1996)* is based on who has the role of a rodent control exacutor.

Rodent control by certified company	**Score**
1. Check of regularity of activities performed by control company	☐
2. Check of bait selection	☐
3. Check of whether bait stations have notices	☐
4. Check of the number of bait stations (boxes)	☐
5. Check of whether they contaminate food	☐
6. Check of whether bait stations (boxes) are regularly inspected	☐
7. Check of whether fumigants are used simultaneously	☐
8. Check of potential hazard to personnel	☐

Figure 2. List filled out by a certified company performing rodent control

The lists (Figures 2 and 3) remain a permanent property of each respective facility. List composition depends on a variaty of factors (Table 2) that are the main indicators of food safety.

Rodent control performed by personnel	**Score**
1. Seek advice before using a poison (there is no use-for-all-purposes pesticide)	☐
2. Map all bait stations (boxes)	☐
3. Regular rodent control	☐
4. Investments in facility maintainance in relation to rodent management	☐
5. Additional measures for rodent management	☐

Figure 3. List filled out by facility presonnel performing rodent control

A book of guidelines for autoinspection by food manufacturers *(Barrett, 1996)* provides an exemplary list to be filled out by personnel of a facility that has not engaged a rodent control company (Figure 3)

Table 2. Vectors and influential factors in facilities, critical control points in facilities A and B, and preventive/monitoring points

Vectors	**Influential factors**	**Critical control points** **CCPA/CCPB**			**Preventive/monitoring points**
Live farm animals		Farm of origin	CCP1	CCP1	Health status Supplier/Reception farm
		Isolation unit	/	/	Blood analysis for specific pathogens Vaccination
		Transfer	CCP2	CCP2	Wash with water and soap, disinfection. Give laxative 2 weeks before transfer
Transport	Feed Livestock Management Maintainance Veterinary doctors/visitors	Internal			Disinfect bathrooms on farm and feeding troughs (clean, disinfect and dry every night)
Dead animals	Carcasses	Transfer		CCP3	Drain bodily fluids, animal burial at livestock cemetary
Humans	Personal higiene	Showering/change of clothes Higiene of hands		CCP4	Use of antimicrobial soaps, shampoos, clothes
	Visit records	Visit history		CCP5	Who/When/Where/Why
Birds	Feces	Doors Windows Other openings	CCP3 CCP4 CCP5	CCP6 CCP7 CCP8	Secure openings
Rodents	Dead Feces	Food Wastes	CCP6 CCP7	CCP9 CCP10	Regular rodent control Transfer of dead rodents Hygiene of food Higiene of wastes
Flies		Ventilation systems	CCP8	CCP11	Ventilation slightly prevents fly entry Use fly traps
Movement of people	Buildings	Personnel/visitors (limited entry)		CCP12	Fences/one-by-one entry/locked doors
		Offices (dining rooms)			Use of antimicrobial cleaning chemicals
	Showers	Movement from pen to pen Movement from			Disinfect footware and clothes upon entry to each room, clear weeds/long grass

		room to room Surrounding area Clean walls/floors	CCP9 CCP10	CCP13 CCP14	around pen area Weekly cleaning with antibacterial chemicals
Pens	Cleaning				
Feed	Composition	Choice of ingredients Storage higiene		CCP15	Purchase from approved suppliers Weekly inspection/cleaning/sterilization
	Complete feed			CCP16	Microbial testing
Air	Wind direction	Distance to nearest farm			Keep legal minimum
Water	Water potability	Potability in pens			
Tools/equipment	Needles/knives/ scissors			CCP17	Disinfect between different operations
Dining containers and cutlery		Dining area			Do not allow contamination with pathogens, special care with meat and eggs

Table 2 presents vectors, pathways of pathogen entry and spreading of contamination inside a facility by: movement of people, their clothes and footware, tools, equipment, packaging material, and dining containers and cutlery; animal feed, its internal distribution and supply by different manufacturers; buildings, barns, air and water as segments in a chain of contamination.

The facilities A and B keep records about the health status of animals entering the farms, which is a responsibility of the veterinary medical service. Quarantine is provided, animals are isolated according to current regulation, inspected in daily visits but, due to their highly risky character, the procedures are marked as $CCP_{1.}$ Data on pathogen check were not presented to us for perusal.

Biosecurity protocols were also defined for drivers and suppliers. Security barriers were found on farm entrances.

Sanitary protocols were not respected, especially not the procedure of sanitary cleaning. An additional CCP_2 risk was detected in the process of animal transfer. Contamination by transport is a source of pathogens both for the facility of origin and facility of arrival.

There had been no repairs of doors, windows and walls, and openings were not secured from bird entry as CCP (A_{345} and B_{678}). Poor ventiation systems and the presence of flies were recorded as $CCPA_8$ and $CCPB_{11.}$

Facility A applies procedures for handling dead animals, procedures for transport of animals and people, which prevents hazards of contamination of food, water and air.

Sanitary inspection and quality management checks are performed at request, which enables early pathogen discovery, as well as omission to make request, which leads to contamination. The surrounding area is cleared of weeds, raw material, broken packaging or other accumulated wastes. However, poor drainage resulting from unfavourable terrain may cause accumulation of filthy water and insect propagation (CCP_{910}). A controlled access zone and video surveillance at entry points should be provided.

Facility B does not have a livestock cemetery, and there is a contamination hazard within it both for people and animals (CCP_{345}). Management rules have not been adjusted to prevent pathogen spreading *(Nielsen et al., 2004).*

Rodent management is important in all rooms throughout the production chain and control should not be expected to be better in a kitchen than in a storage room ($CCPA_{67}$ and $CCPB_{910}$). In both facilities, dead rodents were not removed regularly according to instructions (Table 3).

Table 3. Degree of rodent invasion and estimated population densities in two facilities

Population density estimate		**Facility A**		**Facility B**	
		*Mm**	*Rn**	*Mm*	*Rn*
Relative score	- Feces found	4	4	3	5
	- Transect	4	4	3	5
Absolute score	- Number of active holes	4	4	4	5
	- Damage	4	3	5	5

* *Mm - Mus musculus;* **Rn - Rattus norvegicus*

The analyzed procedures (Tables 1 and 2) led to a need for checking the degree of invadedness *(Hrgović, 1991)* by *Mus musculus* and *Rattus norvegicus* populations in the facilities.

Feces found and transect of *Mus musculus* and *Rattus norvegicus* in facility A were scored 4. In facility B, the score regarding *Mus musculus* was 3, while *Rattus norvegicus* was scored 5, and the Norway rat prevailed in numbers due to more favourable conditions for their propagation, suppressing house mice (Table 3).

The number of active holes of *Mus musculus* in both facilities was scored 4. A tendency was detected of increasing population of *Rattus norvegicus* in facility B (5) and consequential greater damage (5) and contamination of animal products (Table 3).

Biosecurity measures need to be applied in the inspected facilities using prescribed guidelines *(Đedović et al., 2014)* in order to reduce biorisks and strengthen food safety.

Conclusion

- Implementation of the HACCP concept marks a revolutionary advance in livestock production. Adequate implementation and inspection of all requisites secure food quality and reduce food poisoning. Controlled processes ensure that food safety risks be reduced to an acceptible level.

- The prerequisite programme SSOP, cleaning, maintainence and rodent control are essential for securing safe food processes. It is not possible to produce safe food in a facility that is not clean, upkept and free of rodents.

- Hygiene accreditation is viewed by many companies in food industries as an improvement. An external supervisor checks the elements of prerequisite and HACCP programmes and signs a accreditation.

- Rodent control companies must be certified as fully trained. Hitherto practice needs to be changed to impose standards of rodent management in all food-containing rooms. Many companies provide excellent service in that respect.

- Rodent control is not a CCP, it is rather a means used for controlling and monitoring CCPs and its functioning throughout food production processes is expected.

- Speedy changes in world food industries require speedy changes in the rodent management in order to secure first class, transparent and well-documented service.

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Preduslovni programi i kontrola glodara u stočarskoj proizvodnji

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Rezime

Mehanizmi koji doprinose kontroli bezbednosti hrane, životnoj sredini, radnim uslovima za zaposlene i dobrobiti farmskih životinja su preduslovni programi u poljoprivrednoj proizvodnji, odnosno stočarskoj. Dobra poljoprivredna praksa (GAP), dobra proizvođačka praksa (GMP) i sanitarna operativa (SSOP) su preduslovi HACCP programa. Primena ovih programa sprečava kontaminaciju hrane različitim patogenima i čini osnovu za analizu opasnosti i kritične kontrolne tačke.

Jedna od najvažnijih tačaka (SSOP) je kontrola glodara, odnosno kontrola potencijalnih rizika koji mogu biti prouzrokovani njihovom aktivnošću. Sve kompanije za kontrolu glodara moraju biti dobri poznavaoci IPM (integralni menađment glodara), HACCP programa i zahteva COSSA (bezbedna upotreba hemikalija). Operateri kontrole glodara poseduju sertifikat o akreditaciji higijene, kojim se omogućava bezbednost proizvoda. Mada iskustva pokazuju da kompanije za kontrolu glodara ne poštuju propisane standarde i procedure uprkos dugogodišnjem postojanju IPM. Podaci koji se vode su često nepotpuni.

Ovaj rad opisuje značenje preduslovnih programa za bezbednost hrane i HACCP program u tehnološkim procesima proizvodnje. Procenjuje interakciju tehnologije kontrole glodara, štete i mogućnosti kroz preduslove kontrole glodara, karaktera kontrole i posledice loše implementacije HACCP programa. Naglašava se važnost kontrole glodara i zaključujemo da je potrebno usaglasiti promene kao rezultat napretka u tehnologiji hrane.

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NATURAL OCCURRENCE OF DEOXYNIVALENOL IN WHEAT BRAN DURING 2013 AND 2014 IN SERBIAN REGION SREM

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Original scientific paper

Abstract: In this study, a total of 38 samples of wheat bran were collected from two producers from Serbian region Srem. The samples were analyzed for deoxynivalenol (DON) content by using an enzyme-linked immunosorbent assay Ridascreen® Fast DON. Analysis of variance revealed a significant difference between years in DON contents ($p < 0.05$). The occurrence of samples with DON was 35.7% in 2013, with maximum DON content of 99 µg kg^{-1}, and 100% in 2014, with maximum DON content of 1250 µg kg^{-1}. The limit of 8000 µg kg^{-1}imposed by Serbian legislation for DON content was not exceeded in studied samples. The average monthly rainfall for May played a critical role in DON content of samples produced after harvest in 2014.

Key words: deoxynivalenol, wheat bran, Srem

Introduction

Animal welfare continues to be an important issue in the whole world. Therefore the production of safe feed remains one of the most important tasks because the consumption of contaminated feeds by livestock has been associated with a variety of adverse health effects including feed refusal (mainly by swine), reduced weight gain, diarrhea and emesis *(Krska et al., 2001; Kuiper-Goodman, 2002)*. Wheat bran, a by-product of the dry milling of common wheat (*Triticum aestivum* L.) into flour, is one of the major agro-industrial by-products used in animal feeding. It consists of the outer layers (cuticle, pericarp and seedcoat) combined with small amounts of starchy endosperm of the wheat kernel. *(www.feedipedia.org)*. Each year a large number of crops are affected by fungal

invasion. Most of the infection is set in outer layers of the seed, which makes a wheat bran highly contaminated product. The most important agriculturally toxigenic fungi occurring in the moderate climatic zones of North America and Europe are *Fusarium* fungi *(Kos et al., 2003)*. According to the literature, DON is the main *Fusarium* toxic secondary metabolite and its occurrence is also considered to be an indicator of the possible presence of other, more toxic, trichothecenes *(Jajic et al., 2008)*. Although DON is among the least toxic of the trichothecenes, it is the most frequently detected one throughout the world *(Lombaert, 2002)*. According to Serbian legislation maximum permitted level of DON in feed with a moisture content of 12 % in cereals and cereal products is 8000 $\mu g\ kg^{-1}$ *(Službeni glasnik RS, 2014)*.

Serbia is located in moderate continental climate belt. However, variability in precipitation and temperatures and the frequency of extreme events increased over the past few years as was predicted for whole Europe *(Christensen and Christensen, 2003; Falloon and Betts, 2010)*.

Increased crop stress from heavy precipitation results with lower resistance to fungal invasion and increases the occurrence of mycotoxins (*Paterson and Lima, 2011)*.

The aims of this study were: 1. to provide a picture of the occurrence of the mycotoxin DON in wheat bran produced in Serbian region Srem during 2013-2014; 2. to determine a possible effect of precipitation during 2013-2014 on occurrence and level of contamination.

Materials and Methods

Thirty-eight samples of wheat bran were collected from two producers from Serbian region Srem. Fourteen of them after the harvest and milling in 2013 and 24 after the harvest and milling in 2014. The presence of DON was analyzed by enzyme-linked immunosorbent assay (ELISA) method according to R-biopharm Ridascreen® Fast DON SC manual instruction. Limit of detection (LOD) was 75 µg/kg and recovery (R) was 93%.

Chemicals and mycotoxin standards. All reagents were purchased from R-biopharm. Test kit for DON determination contains: 1 microtiter plate with wells coated with capture antibodies against antiDON antibodies, 1 DON standard solution, 1.3 ml 0 ppm (zero standard) in water, 1 peroxidase-conjugated DON, 1 anti-DON antibody, 1 substrate/chromogen, 1 stop solution containing 1 N sulfuric acid and 1 Washing buffer (Salt) for preparation of a 10 mM Phosphate Buffer (pH 7.4) containing 0.05% Tween 20.

Sample preparation. Weigh 5 g of ground sample, put it into a suitable container and add 100 ml of distilled water; blend the sample by ultra-turrax (or

equivalent) for two minutes or shake vigorously for three minutes (manually or with shaker); filter the extract through Whatman No. 1 filter (or equivalent); use 50 µl of the filtrate per well in the test.

Test procedure:
1. Insert a sufficient number of wells into the microwell holder for the standard and samples to be run. Record standard and sample positions.
2. Pipet 50 µl of standard or prepared sample into separate wells; use a new pipette tip for the standard or each sample.
3. Add 50 µl of enzyme conjugate to the bottom of each well.
4. Add 50 µl of anti-DON antibody solution to each well. Mix gently by shaking the plate manually and incubate for 5 min (+/-1) at room temperature (20–25 °C / 68–77 °F).
5. Dump the liquid out of the wells into a sink. Tap the microwell holder upside down onto a clean filter towel (three times in a row) to remove all remaining liquid from the wells. Using a wash bottle or multichannel pipette fill the wells with distilled or deionized water (250 µl per well). Empty the wells again and remove all remaining liquid. Repeat the washing steps two more times.
6. Add 100 µl of substrate/chromogen to each well. Mix gently by shaking the plate manually and incubate for 3 min (+/-0.5) at room temperature (20–25 °C / 68–77 °F) in the dark.
7. Add 100 µl of stop solution to each well. Mix gently by shaking the plate manually and measure the absorbance at 450 nm. Read within 10 minutes after the addition of stop solution.

All obtained data were analyzed using SPSS 15.0 software (SPSS, IBM corporation, USA). Single factor analyses of variance using One-way ANOVA procedure was used.

Results and Discussion

Of the 38 samples analyzed, 76.3 % were contaminated with DON (Table 1). In 2014, 100 % of the samples were contaminated – 64.3 % more than in 2013. Significant increase in both occurrence and level of contamination could be explained by the fact that in 2014 the amount of rainfall in May was 63 % grater than in 2013 (Table 2). These results agree with those described by *Hooker et al. (2002)*, that DON content in grains is highly associated with weather conditions at flowering and after flowering, specifically the positive correlation between DON content and rainfall, with the timing of rainfall being more influential than the amount of precipitation. Similar conclusion was made by *Šlikova et al. (2013)* when analyzing the occurrence of DON in wheat samples in Slovakia in period of

2010 – 2011. These conclusions also agree with previous research made in Serbia. *Jakšić et al. (2012)* reported that 65.3 % of the wheat samples were contaminated with DON in 2012, in a year in which rainfall in May was also above average. In the period 2005-2007 when the amounts of rainfalls in May were within the limits of average *Jajić et al. (2008)* reported that 34.5 % of wheat samples were contaminated with DON.

Table 1. Occurrence of DON in wheat bran in Serbian region Srem in 2013 and 2014

Year	No. of samples	No. of positive samples (%)	Concentration in samples		
			Average ± SD (μg kg^{-1})	Range (μg kg^{-1})	Median (μg kg^{-1})
2013	14	5 (35.7)	93 ±5 .34	87-99	92
2014	28	28(100)	652.16 ± 198.22	343-1250	629.5
Σ2013 2014	38	33(76.3)	555.76 ± 280.15	87-1250	585

Table 2. The rainfall, temperature and relative humidity in Serbian region Srem in May and June 2013 – 2014 *(www.hidmet.gov.rs)*

Year	Month	Rainfall (mm)	Temperature (°C)	Relative humidity (%)
2013	May	119	17.3	71
	June	62	20	74
2014	May	189	16.1	75.9
	June	37.2	20.3	78.2

Conclusion

By analyzing the DON content in samples of wheat bran produced in Serbian region Srem in 2013 and 2014, it was found that the samples produced after the harvest in 2014 were 100% contaminated, which is 64.3% more than after the harvest in 2013. Also, the average level of contamination from 2014 is 7 times grater than in 2013. A relationship was confirmed between the occurrence and levels of contamination and heavy rainfall in May 2014, when the right conditions for the development of DON-producing *Fusarium* were created.

Acknowledgment

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Prirodna pojava deoksinivalenola u stočnom brašnu u 2013. i 2014. godini u regionu Srema u Srbiji

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Rezime

U ovom radu, ukupno 38 uzoraka stočnog brašna prikupljeno od dva proizvođača iz regiona Srema u Srbiji su analizirani na prisustvo deoksinivalenola (DON) primenom imunoadsorpcione enzimske procedure Ridascreen® Fast DON. Analizom varijanse utvrđene su značajne razlike ($p < 0.05$) između godina ispitivanju u sadržaju DON. U 2013. godini ustanovljeno je 35.7% DON pozitivnih uzoraka s maksimalnom koncentracijom od 99 µg kg^{-1}, a u 2014. godini ustanovljeno je 100% DON pozitivnih uzoraka s maksimalnom koncentracijom od 1250 µg kg^{-1}. Sadržaj DON u ispitivanim uzorcima pšeničnih mekinja nije premašio maksimalno dozvoljeni limit (8000 µg kg^{-1}) prema Pravilniku Republike Srbije Prosečne mesečne padavine u maju su imale značajnu ulogu za sadržaj DON u uzorcima proizvedenim posle žetve pšenice u 2014. godini.

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STATUS OF MINERAL NUTRIENTS IN MAIZE GRAIN FROM MONOCULTURE

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Abstract: Nutrition is important factor which provides mineral nutrients to animals. Many mineral nutrients, such as Ca, Mg, Fe, Zn, etc. can't be utilized by to non-ruminants, due to factors which restrain their availability. The aim of this study was to establish effectiveness of different fertilization regimes, like application of farmyard manure, biofertilizer, mineral fertilizers (NPK and Urea) and their combination on grain yield and quality of produced maize grain from the nutritional point of view (including accumulation of P, Ca, Mg, Fe and Zn and ratios between P and other mineral nutrients, as well as Ca/Mg ratio). Combination of manure, biofertilizer and NPK + Urea mineral fertilizers was the most efficient for grain yield increase, together with the accumulation of macro-nutrients: P, Ca and Mg, with decreased ratio between P and other mineral nutrients and Ca/Mg, to some extent. Manure + biofertilizer could partially substitute application of mineral fertilizers, showing the best effect on accumulation and bio-availability of Zn. Urea application was favourable for accumulation of Mg, Zn and particularly Fe, reducing also P/Fe values, indicating better Fe bio-availability. This means that proper combination of organic fertilizer (like farmyard manure), bio- and mineral fertilizers could improve nutritional quality of maize grain considered as non-ruminants feed, even from monoculture.

Key words: bio-availability, fertilization, maize monoculture, mineral nutrients

Introduction

Nutrition is important factor which provides mineral nutrients to animals. They are mainly required in small amounts, depending on the mineral and animal species. Phosphorus is an important for energetic processes in organism, as a part

of ATP, it is also constituent of nucleic acid and is also essential for acid-base balance, bone and tooth formation. Calcium is important for construction and maintenance of bones and normal function of nerves and muscles. Iron is an important component of hemoglobin and cytochromes. Magnesium, zinc, iron, and other micro-elements are important co-factors in the enzymes and are indispensable in numerous biochemical pathways. *Soetan et al. (2010)* emphasized that the interactions between nutrition and diseases is highly present.

Many mineral nutrients, such as Ca, Mg, Fe Zn, etc. can't be utilized by to non-ruminants, due to factors which restrain their availability, such phytic acid. Phytic acid (*myo*-Inositol(1,2,3,4,5,6)hexa*kis*phosphate) is the storage P form in seeds. Excretion of 'phytic acid P' by non-ruminants can contribute to water pollution (*Raboy, 2001*). From this point the ratios of mineral nutrients with P are useful tool in understanding of some complex relations in the plant ionome, giving valuable information about potential bio-availability of some mineral nutrients (*Šimić et al., 2012*; *Dragičević et al., 2013*).

The stability of maize yield is an important factor, particularly if crop is producing at the same field for many years. Disadvantages of such cropping system reflect through increased weed and pest infestation, as well as yield decrease. Monoculture can, also, deteriorate physico-chemical and biological characteristics of soil. On the other hand, maize is one of the crops that are tolerant to continuous growing in monoculture (*Todorović and Božić, 1995*). But, intensive and high yielding crop production considers the application of the measures system which is not short-lived, and it has to show results during the longer period. Results from the long-lasting study conducted in Zemun Polje, showed that grain yield of maize was the lowest in monoculture in 21 year average (6.75 t ha^{-1}), (*Videnović et al., 2007*). Although maize monoculture affects microbiological processes, mainly in poorer soils, high microbial activity could be maintained in rhizosphere of chernozem and other fertile soils (*Kovačević, 2010*). *Adiku et al. (2009)* state that the crop rotation and residue management practices can significantly affect maize performances. Furthermore, application of some measures, like farmyard manure and biofertilizers, such phosphate solubilization microorganisms and plant growth promoting rhizobacteria could improve yielding potential, together with reduction in fertilizer inputs (*Yazdani et al., 2009*). *Ahmad et al. (2008)* also confirmed that application of organic fertilizers and biofertilizers significantly enhanced N and P uptakes with reduced rate of water loss from the soil and increased aggregate stability.

Based on a long-term experimental study, the aim was to establish effectiveness of different cropping measures, like farmyard manure, biofertilizer, mineral fertilizers and their combination on grain yield and quality of produced maize grain from the nutritional point of view.

Material and Methods

Experiment started in Maize Research Institute "Zemun Polje" in 1972 with the aim to evaluate effects of maize growing in continuous monoculture on crop production. The late maturing maize hybrid ZPSC 704 was grown continuously at the same field. New late maturing hybrid ZPSC 684 Ultra was introduced in 2011 in order to conduct perennial grass weed control with the application of cycloxydim. The soil type at the experimental field is slightly calcareous chernozem with 53% sand, 30% silt, 17% clay and soil structure is silty clay loam; with good fertility (pH 6.9; 3.3% of organic matter content; 30.40 kg ha^{-1} N; 44.55 kg ha^{-1} P; 60.57 kg ha^{-1} K, 8494 kg/ha^{-1}Mg and 392.30 kg ha^{-1} Ca) and moderate drainage.

The farmyard manure application is the first factor in the experiment and it has two levels: manure application in 2011 in the amount of 60 t ha^{-1} (M) and without manure application (MØ). The application of microbiological fertilizer Bioplug on decomposition of crop residues is the second factor with two levels: application in amount of 5 l ha^{-1} (BF) and without it (BFØ). Mineral fertilizer application, as the third factor included three variants: F1 - application of NPK fertilizer in autumn (N:P:K= 15:15:15, 1709 kg ha^{-1}) and N in spring (Urea, 320 kg ha^{-1}), F2 - only application of N fertilizer in spring (Urea, 237 kg ha^{-1}) and F3 - without mineral fertilizers.

At the end of vegetative season of 2013, maize was harvested and grain yield was measured and calculated with 14% of moisture. The content of phosphorus, calcium, magnesium, iron and zinc was determined from maize grain. Grain samples were milled and digested with H_2SO_4 + HCl + H_2O_2 mixture. After that P content was determined by the vanado-molybdate colorimetric method, according to *Pollman (1991)*, while Ca, Mg, Fe and Zn were determined by Inductively Coupled Plasma - Optical Emission Spectrometry.

The experimental data of grain yield were statistically processed by analysis of the variance (ANOVA) and analyzed by the LSD-test (5%), while Ca, Mg, Fe and Zn contents were expressed with the standard deviation (SD).

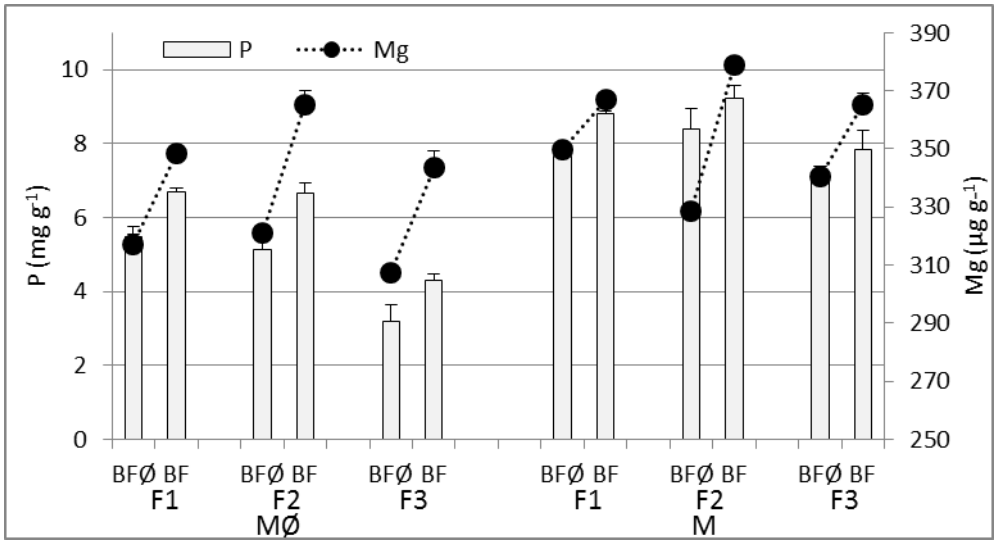
Results and Discussion

The applied fertilizers mainly induced insignificant variations in maize grain yield (Table 1). Only the highest input of mineral fertilizers (F1 - application of NPK + Urea) expressed significant impact on grain yield increase, in average, on the part of the field without manure application. Interaction between

biofertilizer and mineral fertilizers showed significant effect on grain yield increase only in BF + F1 combination at the both fields, with and without manure application (9.97 and 9.90 t ha^{-1}, respectively), contributing to statement that proper nutrition, which include combination of mineral, organic and biofertilizers reflects on grain yield increase (*Bakry et al., 2009*). Irrespective to that that manure increased grain yield in average (about 2%, in relation to part of the treatment without manure application), there was no significant differences between manure application and without it.

Table 1. Maize grain yield influenced by the different fertilization management in monoculture

Treatment	Without farmyard manure			With farmyard manure		
	BFØ	BF	Average	BFØ	BF	Average
F1	7.94	9.90	8.92	7.48	9.97	8.73
F2	6.48	8.31	7.39	7.15	8.83	7.99
F3	6.16	6.38	6.27	7.13	8.51	7.82
Average	7.21	9.10	8.16	7.32	9.40	8.36
LSD 0.05	Biofertilizer	Min. fertilizer	B X MF	Biofertilizer	Min. fertilizer	B X MF
	2.26	2.14	2.19	2.15	2.40	2.42
	Manure 2.30					



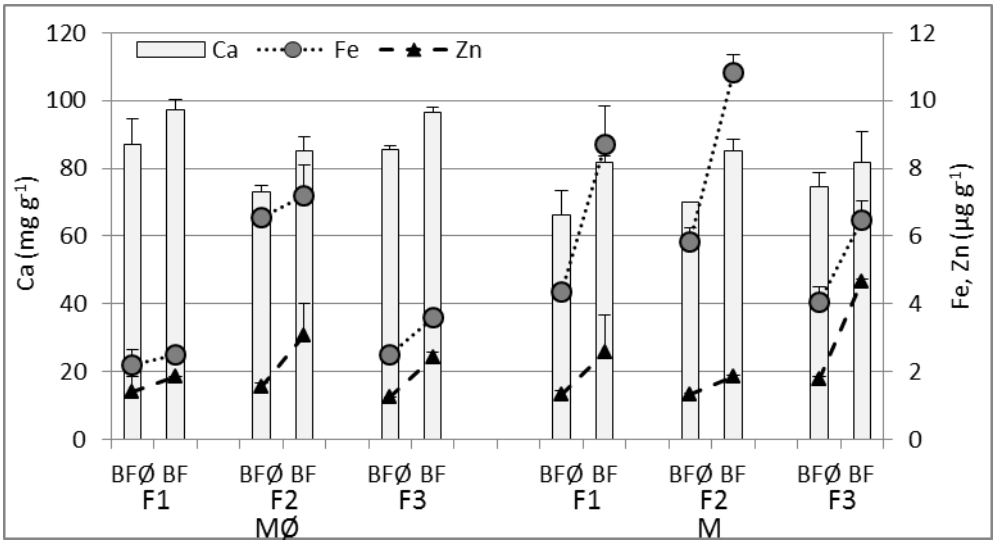


Figure 1. Concentration of P, Mg, Ca, Fe and Zn in maize grain influenced by the different fertilization management in monoculture (MØ - without manure application; M - with manure application; BFØ - without biofertilizer application; BF - with biofertilizer application; F1 - application of NPK fertilizer in autumn and N in spring; F2 - only application of N fertilizer in spring; F3 - without mineral fertilizers).

Along with slight increase in grain yield, manure application involved greater accumulation of mineral nutrients in maize grain, such P, Mg, Fe and Zn on average (36%, 6%, 39% and 14%, respectively, compared to treatment without manure), while average Ca concentration was reduced for about 12% (Figure 1). The positive effect of manure on accumulation of macro- and micro-elements in maize grain was confirmed (*Adeniyan and Ojeniyi, 2003*). Biofertilizer (BF treatment) also showed positive impact on accumulation of mineral nutrients in maize grain. It increased P, Mg, Ca, Fe and Zn concentrations, compared to BFØ treatment, averagely about 22%, 11%, 12%, 15% and 43%, respectively, in treatment without manure application (MØ), as well as about 10%, 8%, 15%, 45% and 51%, respectively, in treatment which included manure application (M), what means that biofertilizer mainly contributed to better Fe and Zn absorption and accumulation in grain, as it was previously confirmed by *Bakry et al. (2009)*. The impact of mineral fertilizers on the accumulation of mineral nutrients in maize grain showed much complex impact. Namely, F1 (application of NPK + Urea) treatment expressed the highest influence on average P and Ca accumulation in maize grain in the field without manure application (MØ), while in the field with manure application (M) it induced greater average Mg accumulation. Subsequently, F2 (Urea) reflected on greater Mg, Fe and Zn accumulation in MØ

treatment, as well as on P and Fe accumulation in M treatment. This contributed to statement that Urea, in combination with Zn containing fertilizers improve Zn absorption and accumulation in grain (*Yadav et al., 2011*). Only in the field with manure application, F3 induced the greatest Ca and Zn accumulation in maize grain, meaning that manure could be efficient source of mineral nutrients for maize, while additional inputs of mineral fertilizers could affect soil microbiological balance and so as mineralization (*Fan et al, 2012*), what could express suppressive effect on absorption of mineral nutrients. Among all combinations of applied treatments, M+F2+BF expressed the highest impact on P, Mg and Fe accumulation (9.24 mg g^{-1}, 378.75 μg g^{-1} and 10.81 μg g^{-1}, respectively), MØ+F1+BF combination expressed the highest impact on Ca accumulation in maize grain (97.19 μg g^{-1}), as well as M+F3+BF combination on Zn accumulation (4.66 μg g^{-1}).

According to *Šimić et al. (2012)* and *Dragičević et al. (2013)*, the ratios of mineral nutrients with P are useful tool in understanding of some complex relations in the plant ionome. Since P majority in maize grain is present in the form of phytic acid, the lowering of ratio between P and other mineral nutrients contribute to potentially better bio-availability of examined mineral nutrients. From this point, some variations between applied treatments exist. Manure mainly increased average values of ratios between P and other mineral nutrients (up to 42%, present at P/Ca ratio), with exception present in P/Fe ratio, which is 17% lower, compared with treatment without manure application (Table 2). As well, biofertilizer application increased ratios between P and other mineral nutrients in MØ treatment (up to 13% in average, present at P/Mg), while in M treatment, biofertilizer mainly decreased ratios between P and other mineral nutrients (down to 38% in average, present at P/Fe), contributing to their better bio-availability. When application of mineral fertilizers is considered, they mainly increased ratios between P and other mineral nutrients, with minor variations between F1 and F2 treatments. Only F2 treatment contributed to reduce of P/Fe values, indicating better Fe bio-availability. Parallel with poorer accumulation of mineral nutrients in maize grain, present in combination of treatments without any type of fertilizer application (MØ+BFØ+F3), the lowest values of ratios between P and other mineral nutrients were also present. However, manure and biofertilizer application in combination with F3 treatment, along with better accumulation of mineral nutrients (when compared to MØ and BFØ) showed the lowest value of P/Zn ratio (3558.8) among all applied treatments. Considering Ca/Mg ratio, as a trait important for osteoporosis (*Rude and Gruber, 2004*), it is important to underline that its value slightly varied among applied treatments. It was increased by biofertilizer application, and decreased by manure and mineral fertilizers application, having the lowest value of 0.189 in M+BPØ+F1 combination.

Table 2. Molar ratio between P, Ca, Fe and Zn and mass ratio between Ca and Mg influenced by the different fertilization management in monoculture.

Treatment			P/Mg	P/Ca	P/Fe	P/Zn	Ca/Mg
MØ	F1	BFØ	13.56	81.24	4511.8	8180.8	0.275
		BF	15.08	89.16	4829.8	7590.3	0.279
	F2	BFØ	12.58	91.09	1421.1	6954.8	0.228
		BF	14.34	101.59	1674.1	4600.1	0.233
	F3	BFØ	8.15	48.22	2301.5	5389.2	0.279
		BF	9.85	57.74	2162.0	3732.0	0.281
Average			12.26±1.07	78.17±8.38	2816.7±1810.8	6074.5±1569.6	0.26±0.027
M	F1	BFØ	17.17	149.44	3176.1	12277.7	0.189
		BF	18.86	139.86	1823.2	7236.5	0.222
	F2	BFØ	20.04	155.00	2601.2	13487.1	0.213
		BF	19.14	140.59	1540.1	10610.4	0.224
	F3	BFØ	16.64	125.10	3204.8	8498.0	0.219
		BF	16.86	124.53	2187.9	3558.8	0.223
Average			18.12±1.20	139.09±7.29	2422.2±744.4	9278.1±2713.9	0.215±0.016

Conclusion

Beside the increase of maize grain yield, combination of farmyard manure and biofertilizer expressed the highest impact on absorption and accumulation of all examined mineral nutrients in grain, with decrease of ratios between P and other mineral nutrients, increasing their bio-availability to some extent. This combination could partially substitute application of mineral fertilizers, showing the best effect on accumulation and bio-availability of Zn. Additional input of NPK + Urea mineral fertilizers was connected to increased grain yield in the highest degree, as well as to the accumulation of macro-nutrients: P, Ca and Mg, with decreased ratio between P and other mineral nutrients and Ca/Mg, to some extent. Urea application was favourable for accumulation of Mg, Zn and particularly Fe, reducing also P/Fe values, indicating better Fe bio-availability. This means that proper combination of organic fertilizer (like farmyard manure), bio- and mineral fertilizers could improve nutritional quality of maize grain considered as non-ruminants feed, even in monoculture.

Acknowledgment

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Status mineralnih nutrijenata u zrnu kukuruza gajenog u monokulturi

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Rezime

Ishrana životinja je važan faktor kojim se obezbeđuju mineralni nutrijenti. Mnogi mineralni nutrijenti, kao što su Ca, Mg, Fe, Zn i dr. ne mogu biti iskorišćeni od strane nepreživara, zahvaljujući faktorima koji ograničavaju njihovu pristupačnost. Cilj ovog istraživanja je da se utvrdi efikasnost različitih režima đubrenja, kao što je upotreba stajnjaka, mikrobiološkog i mineralnih đubriva (NPK i Urea) i njihovih kombinacija na prinos zrna i njegov kvalitet, sa nutritivne tačke gledišta (uključujući nakupljanje P, Ca, Mg, Fe i Zn i odnose između P i ostalih mineralnih nutrienata, kao i odnos Ca/Mg). Kombinacija stajnjaka, mikrobiološkog i mineralnih đubriva NPK + Urea je bila najefikasnija u povećanju prinosa zrna, zajedno sa većim nakupljanjem maroelemenata: P, Ca i Mg, uz smanjenje odnosa između P i ostalih nutrienata, kao i Ca/Mg odnos u izvesnoj meri. Kombinacija stajnjak + mikrobiološko đubrivo može delimično da zameni upotrebu mineralnih đubriva, a pokazala je i najbolji efekat na akumulaciju i pristupačnost Zn. Primena Uree se pokazala kao najpovoljnija za nakupljanje Mg, Zn i posebno Fe, takođe smanjujući P/Fe odnos što ukazuje na veću pristupačnost Fe. To znači da adekvatna kombinacija organskih đubriva (kao što je stajnjak), mikrobioloških i mineralnih đubriva može da poboljša kvalitet zrna kukuruza namenjenog ishrani nepreživara, čak i u monokulturi.

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EFFECTS OF ALFALFA AND RED CLOVER CULTIVARS ON PROTEIN FRACTIONS BY CNCPS SYSTEM OF ANALYZIS

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Abstract: Legumes have a high nutritional value, but the protein of temperate forage legumes is characterized by rapid and extensive degradation in the rumen. The rate and degree of ruminal protein degradation of feeds is an important consideration in the formulation of rations for dairy cattle. The object of the present study was to quantify the main CP fractions of different cultivars of alfalfa and red clover at the mid bloom stage of growth in the first cut. Two cultivars of alfalfa - Serbian *cv* K 28 and American *cv* G+13R+CZ and red clover – tetraploide *cv* K 32 and diploide *cv* K 39, was taken on May 21th at mid-bloom stage in the first cut. Comparing alfalfa and red clover means, alfalfa was higher in CP, SolP, NPN, PA and PB_1, but lower in NDICP, ADICP, IP, TP, PB_2, PB_3 and PC, indicating large and potential differences in rumen CP degradation characteristics between alfalfa and red clover. The slowly degradable PB_3 fraction associated with the plant cell wall and the intermediately degradable PB_2 fraction were higher in red clover than in alfalfa. In general, the chemical CP fractionation provides, in addition to the classical characteristics such as energy or fibre content, further information to achieve a better evaluation of the quality of forage legume species. Moreover, the present study provides valuable data for the feed formulation systems, which would aid in formulating diets such that decreased nitrogen excretion occurs by lactating dairy cows.

Key words: alfalfa, CNCPS, protein fractions, red clover

Introduction

In temperate regions, white clover is the predominant species used for grazing, whereas red clover and alfalfa are cultivated mainly for cutting and silage making. These forage legumes represent a major protein source for ruminants and are a major constituent of low-input farming systems. Although legumes have a high nutritional value, the protein of temperate forage legumes is characterized by rapid and extensive degradation in the rumen.

The rate and degree of ruminal protein degradation of feeds is an important consideration in the formulation of rations for dairy cattle (NRC, 1989). Forage provide much of the protein consumed by dairy cattle, and the degradation of forage protein varies, depending on plant species, stage of maturity, conservation method and season (Kohn and Allen, 1995).

Ruminal degradation of forage protein depends on rate of degradation of individual proteins in the rumen and on rate of passage of those proteins from the rumen. Both of these factors may be influenced by protein associations with other feed components and by availability of peptide bonds to enzymatic attack. Separation of feed proteins based on solubility may have practical significance for degradation of ruminal protein, because ultimately the solubility of a protein depends on the microenvironments, with which it associates, and this association may alter the availability of peptide bonds or the passage rate of the protein.

There is currently increasing interest in forage legume species that undergo reduced proteolysis during ensiling and reduced protein degradation in the rumen. In this context, legume species containing secondary plant compounds, which reduce the protein degradability, are of particular relevance. The protein of tannin-containing species, such as birdsfoot trefoil show lower ruminal degradability compared to the tanin-free alfalfa (Broderick and Albrecht, 1997; Julier et al., 2003). In contrast, red clover has only low levels of condensed tannins (Jackson et al., 1996; Grabber 2009), but the protein degradation rate of red clover is also less that of alfalfa (Krawutschke et al., 2013). The improved protein utilization of red clover by lactating dairy cows compared to alfalfa is generally attributed to the polyphenol oxidase (PPO) activiy in red clover (Broderick et al., 2001, 2004). In contrast, effects on N-use efficiency, such as lower N contents in milk, faeces and urine N excretion, were not observed in recent work when fresh red clover with different PPO activities was fed to dairy cows (Lee et al., 2009).

The object of the present study was to quantify the main CP fractions of different cultivars of alfalfa and red clover at the mid bloom stage of growth in the first cut.

Material and methods

The experiment was designed as a factorial trial, by randomized block system in three replicates. Two cultivars of alfalfa - Serbian *cv* K 28 and American *cv* G+13R+CZ and red clover – tetraploide *cv* K 32 and diploide *cv* K 39, was taken on May 21th at mid-bloom stage in the first cut. Dry matter was determined by drying out samples at 65º C and grinding and sieving them to 1 mm particle size. The samples were dried in an oven of 105º C for 6 h for dry matter determination.

The CP of the samples was determined using Kjeldahl method. The NPN, NDICP, ADICP, SolP, TP and IP were determined by Licitra et al. (1996). The CP, NPN, SolCP, NDICP, ADICP, TP and IP were calculated as follows:

CP = Total N x 6.25

NPN = (Total CP – Residusl CP_{NPN})/CP x 1000

SolCP = (Total CP – Residual CP $_{SolCP}$)/CP x 1000

ADICP = Residual CP_{ADICP}/CP x 1000

NDICP = Residual CP_{NDICP}/CP x 1000

TP = Residual CP_{NPN}/CP x 1000

IP = Residual CP_{SolCP}/CP x 1000

Where, CP is the crude protein, NPN - non-protein nitrogen (g kg^{-1} CP); SolCP, the soluble crude protein (g kg^{-1} CP); NDICP, the neutral detergent insoluble crude protein (g kg^{-1} CP); ADICP, the acid detergent insoluble crude protein (g kg^{-1} CP); TP – true protein (g kg^{-1} CP) and IP – insoluble crude protein (g kg^{-1} CP).

The CNCPS crude protein fractions of the samples, PA, PB, PB_1, PB_2, PB_3 and PC were calculated based on CP, NPN, SolCP, NDICP, ADICP contents of samples according to Sniffen et al. (1992).

PA = NPN

PB_1 = SolCP – NPN

PB_2 = CP – SolCP – NDICP

PB_3 = NDICP – ADICP

PB = 1000 – PA – PC

PC = ADICP

Where, PA refers to the non-protein nitrogen (g kg^{-1} CP); PB_1, the rapidly degraded crude protein (g kg^{-1} CP); PB_2, the intermediately degraded crude protein (g kg^{-1} CP); PB_3, the slowly degraded crude protein (g kg^{-1} CP) and PC, the bound crude protein (g kg^{-1} CP).

Data were processed by the analysis of variance in a randomized block design. Effects were considered different based on significant ($P< 0.05$) F ratio.

Results and discussion

In models designed to assess utilization of dietary protein by ruminants, it is assumed that most of the soluble protein (PA and PB_1) is completely degraded in the rumen, and varying proportions of the insoluble fractions (PB_2, PB_3 and PC) escape ruminal degradation depending on the interactive effect of digestion and passage (Sniffen et al., 1992). Because various protein fractions differ in rate and extent of ruminal degradation, the proportions of these different protein fractions in feedstuffs are believed to influence the amounts of ruminally degraded and escape protein consumed by animals (Elizalde et al., 1999). Table 1 illustrates the quantitative effect of variety of alfalfa and red clover on the proportion of protein (NDICP, ADICP, TP, NPN, SolP and IP) and Table 2 illustrates the protein fractions by CNCPS (PA, PB_1, PB_2, PB_3 and PC).

Table 1. Content of crude protein fractions in alfalfa and red clover, g kg^{-1} CP

Cultivars	CP	NDICP	ADICP	IP	SolCP	TP	NPN
K – 28	160.8^{b}	224.2^{a}	93.6^{b}	501.9^{b}	498.0^{a}	570.8^{b}	429.2^{a}
G+13R+CZ	177.5^{a}	134.2^{b}	107.7^{a}	544.8^{a}	455.2^{b}	578.4^{a}	421.6^{b}
Means	**169.2**	**179.2**	**100.7**	**523.4**	**476.6**	**574.6**	**425.4**
K - 32	164.8^{a}	162.0^{b}	89.7^{b}	609.4^{b}	390.5^{a}	643.7^{ns}	365.7^{ns}
K - 39	150.8^{b}	314.5^{a}	115.4^{a}	713.9^{a}	286.1^{b}	640.2^{ns}	359.7^{ns}
Means	**157.8**	**238.3**	**102.6**	**661.7**	**338.3**	**641.9**	**362.7**

Different letters denote significantly different means ($P< 0.05$)

Comparing alfalfa and red clover means, alfalfa was higher in CP, SolP, NPN, PA and PB_1, but lower in NDICP, ADICP, IP, TP, PB_2, PB_3 and PC, indicating large and potential differences in rumen CP degradation characteristics between alfalfa and red clover. The results indicate that alfalfa had a highly rapidly degradable NPN fraction (Table 1). Therefore, the true protein as a percentage of CP in alfalfa was lower than in red clover (574.6 *vs* 641.9 g kg^{-1} CP).

The slowly degradable PB_3 fraction associated with the plant cell wall and the intermediately degradable PB_2 fraction were higher in red clover than in alfalfa (Table 2). The protein fractions of alfalfa in this study differed from the tabular values in NRC (2001). Fraction PA was higher (425.4 *vs* 278 g kg^{-1} CP), and fraction PC was higher (100.6 *vs* 62 g kg^{-1} CP) that the tabular values in NRC (2001).

Comparing the two varieties of alfalfa, K-28 was higher in NDICP, SolP and NPN, as well as PA, PB_1 and PB_3 fractions of crude protein, but lower in CP,

ADICP, IP, TP, PB_2 and PC fraction of crude protein. Comparing the two varieties of red clover (tetraploide *vs* diploide), there were no differences in TP and NPN and PA fraction of crude protein. Tetraploide cultivar of red clover was higher in CP, SolP and PB_1 and PB_2 fractions of crude protein but lower in NDICP, ADICP, IP and PB_3 and PC fraction of crude protein.

Table 2. Content of CP fractions in alfalfa and red clover by CNCPS, g kg^{-1} CP

Cultivars	PA	PB_1	PB_2	PB_3	PC
K – 28	429.2^{a}	68.7^{a}	271.0^{b}	137.2^{a}	93.6^{b}
G+13R+CZ	421.6^{b}	33.5^{b}	410.6^{a}	26.6^{b}	107.6^{a}
Means	**425.4**	**51.1**	**340.8**	**81.9**	**100.6**
K - 32	365.7^{ns}	25.3^{a}	447.3^{a}	72.4^{b}	89.7^{b}
K - 39	359.7^{ns}	0.0^{b}	399.4^{b}	199.2^{a}	115.4^{a}
Means	**362.7**	**12.7**	**423.4**	**135.8**	**102.6**

Different letters denote significantly different means (P< 0.05)

However, the study by Elizalde et al. (1999) showed that fraction PA was not affected by forage species or maturity, with an average of 22.6% across forages and maturity dates for fresh alfalfa, bromegrass and tall fascue. A large difference in PA content due to the experimental procedure has been reported by Licitra et al. (1996). Sniffen et al. (1992) found that the fraction PB_2 was the largest CB_2 fraction in fresh alfalfa pastures, with a mean value of 41% of the total CP. Elizalde et al. (1999) reported a PB_2 value of 51.6% for fresh alfalfa, 42.5% for fresh bromegrass and 48.5% for fresh tall fascue. The extension protein of the plant cell wall is assumed to be a dietary protein slowly degraded in the rumen but digested in high proportion in the small intestine (Sniffen, 1992). The fraction PB_3 of alfalfa in this study was 81.9 g kg^{-1} CP and this is contrast to the results obtained by Elizalde et al. (1999), who reported that this value was 3% CP in fresh alfalfa.

Conclusion

In conclusion, the proportion of the CP fractions of forage legume species varies between varieties. From the point of view of ruminant nutrition, red clover showed advantages for the CP fractions A and B_3 in comparison with alfalfa. Alfalfa had a higher CP concentration, but its protein is characterized by rapid and extensive degradation in the rumen. Fraction B_2 was the largest CP fraction in all forages. This fraction was greater in red clover than in alfalfa.

In general, the chemical CP fractionation provides, in addition to the classical characteristics such as energy or fibre content, further information to achieve a better evaluation of the quality of forage legume species. Moreover, the present study provides valuable data for the feed formulation systems, which would aid in formulating diets such that decreased nitrogen excretion occurs by lactating dairy cows.

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Uticaj različitih sorti lucerke i crvene deteline na CNCPS proteinske frakcije

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Rezime

Pored toga što se leguminoze odlikuju visokom hranljivom vrednošću, njihovi proteini se odlikuju brzom i obimnom degrdacijom u buragu. Stopa i stepen degradacije proteina hrane u buragu je značajan faktor u formulisanju obroka za krave muzare. Cilj ovih istraživanja je bio da se odredi sadržaj proteinskih frakcija različitih sorti lucerke i crvene deteline u fenofazi sredine cvetanja tokom prvog otkosa. Dve sorte lucerke, domaća K-28 i američka G+13R+CZ i dve sorte crvene deteline, tetraploidna K-32 i diploidna K-39 su pokošene 21. maja sredinom cvetanja u prvom otkosu. Upoređujući lucerku i crvenu detelinu, lucerka se odlikovala većim udelom CP, SolP, NPN, PA i PB_1, ali manjim udelom NDICP, ADICP, IP, TP, PB_2, PB_3 i PC ukazujući na potencijalne razlike u brzini razlaganja proteina između lucerke i crvene deteline. Sporo razgradiva B_3 frakcija kao i B_2 frakcija su bile veće u crvenoj detelini nego u lucerki. Uopšteno, sadržaj sirovih proteina pored klasičnih karakteristika kao što su energetska vrednost ili sadržaj vlakana obezbeđuju dodatne informacije za bolju ocenu kvaliteta leguminoznih vrsta. Štaviše, ova istraživanja pružaju validne podatke za balansiranje proteinskih frakcija, što će biti predmet budućih istraživanja.

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COMMON BEAN (*Phaseolus vulgaris* L.) GROWTH PROMOTION AS AFFECTED BY CO-INOCULATION WITH *Rhizobium* AND RHIZOBACTERIA

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Original scientific paper

Abstract: A field experiment was performed to evaluate the effects of inoculation of the common bean (*Phaseolus vulgaris* L.) with *Rhizobium phaseoli* and different rhizobacteria on common bean grain and dry plant residue yield and their nitrogen content. Inoculation was performed with *Rhizobium phaseoli* strain 110 and strains of genera *Pseudomonas* (strains P59 and LG), *Bacillus* (strains BB and SNji), *Azotobacter* (strains AC and AZ) and *Enterobacter* (strain E1) at the time of sowing. There were 9 different treatments and the control without inoculation and fertilization. Improved common bean grain yield and nitrogen content in inoculated treatments were noted in respect to control plants and/or inoculation with *Rhizobium* alone. The increase in dry plant residue yield ranged between 25% with *Rhizobium* single inoculation and from 12% (*Rhizobium* + *Bacillus* BB) and 33% (*Rhizobium* + *Pseudomonas* p59 and *Azotobacter* AZ) in co-inoculation over control. In some treatments co-inoculation of rhizobacterial strains with rhizobium increased dry plant residue yield over rhizobium inoculation and control indicating positive interactions with *Rhizobium*. The increase in dry plant residue yield ranged between 2% and 28% in co-inoculated treatments over control. In vitro screening for plant growth-promoting traits showed phosphate solubilization, IAA, ammonia and siderophore production by particular strains. Multiple plant growth-promoting traits of some strains may be associated with its ability to improve common bean growth and nitrogen uptake efficiently. The results confirm the possibility of application of rhizobia and rhizobacteria as inoculants in agricultural systems, replacing environmentally unfriendly chemical fertilizers.

Key word: common bean, grain, residues, inoculation, nitrogen

Inoculation

Beans are the most important grain legumes for direct human consumption in the world. Beans residues, such as dried pods and stems can be used as fodder (*Wortmann, 2006; CNC, 2004*). Bean crop residues can be fed fresh to livestock. The average yield of dried beans is 0.5-1.5 t/ha, but yields up to 2.8-5 t/ha have been reported (*Wortmann, 2006*). A yield of 1.6 kg green biomass/m² (about 16 t green biomass/ha) has been reported (CNC, 2004). Common bean can establish a nitrogen-fixing symbiosis with *Rhizobium pha*seoli and fix atmospheric nitrogen of the benefit to the plant. Symbiotic nitrogen fixing potential in common bean is considered to be low in comparison with other legumes, but it is reported to have fixed up to 125 kg N/ha. Besides N_2 fixation, rhizobial inoculation of legumes could have positive effects on some macro and micronutrient contents in plants and overall plant growth (*Howell et al. 1987; Bambara and Ndakidemi, 2010; Ndakidemi et al. 2011*). Available reports indicate improved legumes yield, health and nodulation when co-inoculated with growth-promoting rhizbacteria (PGPR), compared to inoculation with rhizobium alone (*Valverde et al., 2006; Shweta et al., 2008; Yadegari et al., 2010*). Common beans can be mixed with small grains in order to increase the protein content of silage (*Linn et al., 2002*). Therefore, inoculation of common bean with PGP rhizobacteria different than rhizobium can have beneficial effect on both common bean and main crop. Organic beans are produced and processed without the use of synthetic fertilizers and pesticides. The increasing demand for healthy food and the need for environmental and economic sustainability of agricultural production organic farming is being promoted worldwide. Therefore, agricultural researchers are challenged to develop such systems

The aim of the research was to investigate the possibility of common bean cultivation only with *Rhizobium phaseoli* and different rhizobacteria co-inoculation, evaluate the effects of co-inoculation on grain and common bean residue yield in field conditions.

Materials and Methods

The trial was set up in 2010 at Ratare village, Serbia (20˚7'15.5"E and 44˚39'0.3"N) on fluvisol (FAO, 2006) with clayed loam texture and the following granulometric content and chemical properties: sand 31.3%, silt 32.3%, clay 36.4%, pH 6.95 (in KCl), organic matter 4.23%, $CaCO_3$ 0.42%, N% 0.22, NH_4-N + NO_3-N 25 mg kg^{-1}, P 34.73 mg kg^{-1}, K 267.27 mg kg^{-1}. The average monthly temperature during the growing period (from March to October) was 17.3°C with maximum in July 24.4°C and June 24.8°C, while total amount of rainfall was 865.5

mm. In the past 10 years legumes have not been grown on the experimental field. Before sowing and during the vegetation, neither mineral manure was added, nor pesticides. Strains *Rhizobium phaseoli* 110, *Bacillus megaterium* SNji, *Enterobacter* sp. E1, *A. chroococum* strains AC and AZ, as well as *Pseudomonas* sp. strains P59 and LG from the Collection of the Institute of Soil Science were used for the inoculation of common bean (variety Biser). *Bacillus* and *Enterobacter* strains and *Pseudomonas* strains were cultivated for 24h in nutrient broth medium and King B medium, respectively. *S. meliloti* strain was cultivated in yeast mannitol broth (YMB) for 48h while *Azotobacter* strains were cultivated in N free mannitol broth for 72h.The culture of 40 ml of each single strain was mixed with 100 g sterile ground peat and after a 15 day incubation period, single inoculums consisted of approximately 10^9 bacteria g $peat^{-1}$ were obtained. Common bean seeds were inoculated either with *S. meliloti* or rhizobacterial strains alone, or by mixing rhizobium inoculums with *Bacillus*, *Pseudomonas*, *Enterobacter*, or *Azotobacter* inoculums in a ratio of 1:1.

The trial was designed with 8 inoculated treatments, treatment with mineral fertilizer N 60 kg/ha, P 100 kg/ha and K 100 kg/ha, and control without mineral N fertilizer and inoculation (Ø). The experiment was laid out in completely randomized design in three replicates. Each plot was planted in 15 rows of 2 m length with 20 cm inter-row spacing according seed rate of 50 kg ha^{-1}. Grain and empty pods were harvested in two cuts, and in the second cut the complete plants were harvested. Plant shoots were dried in an oven at 70˚C to constant weight and the average dry weight per plot was calculated. The percentage of N was determined from dried and ground grain and plant samples using the CNS analyzer (CNS analyzer, Vario model EL III, Elemental Analysis systems GmbH, Hanau, Germany). The data were statistically processed by the LSD and Duncan test using the statistical program COSTAT.

Results and Discussion

Improved common bean grain and dry plant residue yield and nitrogen content were noted in inoculated treatments in respect to control plants Ø (Table 1, Figure 1). In some cases co-inoculation of rhizobacterial strains with rhizobium increased yield over rhizobium inoculation indicating positive interactions with rhizobium. The increase in grain yield ranged between 25% in single inoculation *Rhizobium* and from 5% (*Rhizobium* + *Pseudomonas* LG) to 32% (*Rhizobium* + *Azotobacter* AZ) and 33% (*Rhizobium* + *Pseudomonas* p59) in co-inoculation over control Ø. In some cases this increase is not statistically significant.

Table 1. Common bean grain and dry plant residue yield under the inoculation with *Rhizobium* and co-inoculation with rhizobacteria (g/plot)

Treatment	Biomass yield g/plot	Grain yield g/plot	Harvest index	N% in dry plant residues	N% in grain
Rhizobium 110	187.93[d]	256.82[bc]	0.58	2.27	3.45[ab]
Rhizobium + *Azotobacter* AC	234.12[cd]	259.39 [bc]	0.53	2.40	3.67[a]
Rhizobium + *Enterobacter* E	221.55[cd]	246.45 [bcd]	0.53	2.34	3.33ab
Rhizobium + *Bacillus* BB	257.31[bc]	230.20 [bcd]	0.47	2.31	3.44[ab]
Rhizobium + *Pseudomonas* P59	278.64[ab]	273.36 [ab]	0.50	2.33	3.57[a]
Rhizobium + *Azotobacter* AZ	132.36[e]	270.14 [ab]	0.67	2.17	3.74[a]
Rhizobium + *Bacillus* SNji	253.34[bc]	244.66 [bcd]	0.49	2.22	3.42[ab]
Rhizobium + *Pseudomonas* LG	198.71[d]	215.29 [cde]	0.52	2.20	3.52 [a]
Ø	217.58[cd]	205.43 [d]	0.49	2.23	3.12b
NPK	304.23[a]	307.78 [a]	0.50	2.37	3.64ab
LSD 0.05	42.77	43.53		0.31ns	0.29

Ø non-inoculated control; NPK- treatment with NPK fertilizer; Means in a column followed by the same letter are not significantly different, according to Duncan's multiple range test at the 5% level ($p \leq 0.05$).

The treatments with *Rhizobium*, *Rhizobium* + *Azotobacter* AC, *Rhizobium* + *Azotobacter* AZ and *Rhizobium* + *Pseudomonas* p59 realised significantly higher grain yield than control Ø, while two later there did not significant differ from NPK treatment. Harvest index ranged between 0.44 and 0.67 which is similar to the previous reports.

Common bean residues (dry matter of shoots and pods) significantly increased only in the treatment *Rhizobium* + *Pseudomonas* P59 compared to control plants, and also this treatment did not significantly differ from NPK treatment. Because fallen leaves, flowers, pods, and seeds were not collected during the growing season and harvest, the dry plant residue yield may be up to 15% lower than the real values. Increase in residue yield was from only 2% up to 28% (*Rhizobium* + *Pseudomonas* P59). Significant decrease in residue yield was noted in the treatment with *Rhizobium* + *Azotobacter* AZ in which the significant increase in grain yield was noted. There was significant increase in N percentage in grain in some of co-inoculated treatments compared to the control, while in dry plant residue N percentage there were no differences among all treatments. In fertilized treatment (NPK treatment) there were significant and the highest percentage in both grain and residue yield (Figure 1).

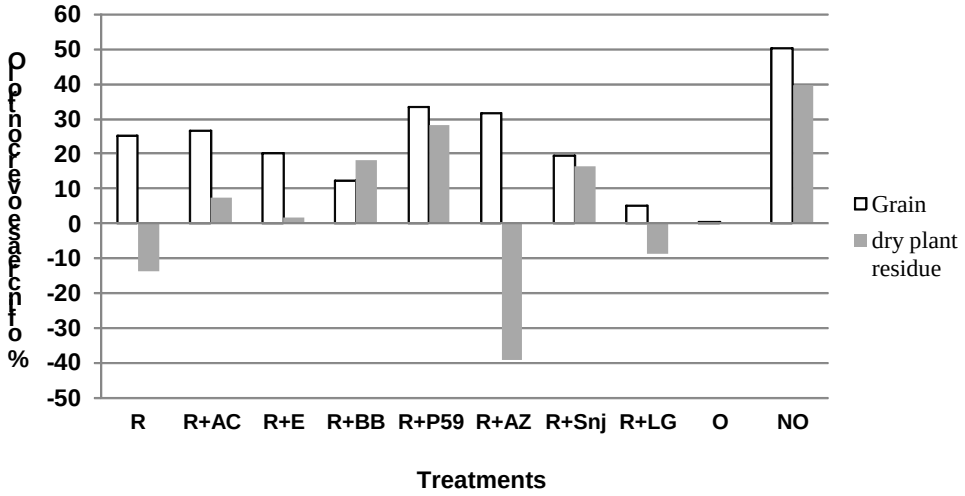

Figure 1. Common bean grain and dry plant residue yield increase in respect to control Ø

Similarly to our study, significant positive effect of legumes co-inoculation with rhizobium and rhizobacterial isolates (*Bacillus*, *Azospirillum* sp., etc.) on different growth parameters (plant biomass, accumulation of protein) as well as grain yields were previously reported (*Hungria et al., 2013; 2015; Itzigsohn et al., 1993*). In contrast, there are also the studies showing the absence of positive effect of co-inoculation in respect to single rhizobium inoculation which is also the case with some rhizobacterial strains in our study (*Rosas et al., 2006*). The absence of the co-inoculation effect of some rhizobacterial strains, could be due to its lower density in co-inoculation or the consequence of strains competition with each other or with other soil bacteria for environment (nodules) and nutrients (*Mrabet et al., 2006*). Although, many studies evaluated the effects of PGPR on the early aspects of symbiotic relationship mainly in laboratory or greenhouse conditions, for common bean there are evidences about the co-inoculation effect of rhizobia and *Azospirilum* on yield increase in the field condition in multi-year trial *(Hungria et al., 2013; 2015*). The good response of common bean to mineral N fertilizer observed in our study is in agreement with the previous reports (*Vargas et al., 2000)* and also confirms the poorer nitrogen fixing ability of beans.

All the strains investigated in this study showed two or more mechanisms which might be involved in plant growth promotion (*Stajković et al., 2009; 2011*). Most of the applied rhizobacteria belong to nitrogen-fixing and phosphorus-solubilizing bacteria that may be important for plant nutrition by increasing N and

P plant uptake. All strains used in this study produced IAA, phytohormone, which is often directly connected with rhizobacterial potential to stimulate plant growth (*Spaepen et al., 2008*). Possibly, plant growth promotion is the results of simultaneous activity of different mechanisms.

Conclusion

The presented results showed a significant positive effect of inoculation with rhizobium and different rhizobacteria on grain and plant residue yield of common bean compared to the control and/or the inoculation with rhizobium alone. Co-inoculation of common bean with rhizobium and some rhizobacterial strains increased yield over rhizobium inoculation indicating positive interactions with rhizobium. The results confirm the benefit of application of rhizobia and rhizobacteria as inoculants in agricultural systems as an alternative to chemical fertilizers.

Acknowledgment

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Poboljšanje rasta pasulja (*Phaseolus vulgaris* L.) inokulacijom sa *Rhizobium* i rizobakterijama

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Rezime

U ovom radu je ispitan efekat inokulacije semena pasulja (*Phaseolus vulgaris* L.) sa *Rhizobium phaseoli* i različitim rizobakterijama na prinos zrna i ostataka suve biljne mase pasulja u poljskim uslovima. Inokulacija je izvršena sojem *Rhizobium phaseoli* 110 i sojevima rodova *Pseudomonas* (sojevi P59 i LG), *Bacillus* (sojevi BB i SNji), *Azotobacter* (sojevi AC i AZ) i *Enterobacter* (soj E1) u vreme setve. Ukupno je bilo 9 tretmana i kontrola bez inokulacije i bez djubrenja. Uvećan prinos zrna pasulja i sadržaja azota u zrnu u inokulisanim tretmanima je zabeležen u odnosu na kontrolne biljke i/ili biljke inokulisane samo sa *Rhizobium*. Prinos zrna pasulja je bio povećan za 25% kod inokulacije samo sa *Rhizobium* i od 12% (*Rhizobium* + *Bacillus* BB) do 33% (*Rhizobium* + *Pseudomonas* p59 i

Azotobacter AZ) kod ko-inokulacije u odnosu na kontrolne biljke. U nekim tretmanima ko-inokulacija rizobakterijalnih sojeva sa rizobijumom je povećala prinos ostataka suve mase pasulja u odnosu na inokulaciju sa rizobijumom i kontrolu, ukazujući na njihovu pozitivnu interakciju. Ovo povećanje se kretalo od 2% do 28% u odnosu na kontrolu. Povećanje prinosa zrna i ostataka suve biljne mase pasulja pod uticajem rizobakterija može biti rezultat jednog ili više mehanizama delovanja baktrija koje poboljšavaju biljni rast. Svi sojevi rizobakterija korišćeni u ovom radu su pokazali neke mehanizama poboljšanja biljnog rasta kao što su solubilizacija fosfata, produkcija auksina (IAA), amonijaka ili sidrofora. Rezultati ovog rada potvrdjuju mogućnost primene rizobija i rizobakterija kao inokulanata u poljoprivredi, kao zamenu mineralnim djubrivima.

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PHYTOPLANKTON STRUCTURE IN THE DRINKING WATER SOURCE DURING A CYANOBACTERIAL BLOOM EPISODE

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Original scientific paper

Abstract: Cyanobacteria or blue-green algae can cause major water quality problems through the effect of toxins which pose threats for humans and animals.The present study described some aspects of the structure of the phytoplankton community in Ćelije Reservoir and its basins during a brief episode of cyanobacterial bloom. In the surface layer of water (in all of three basins), the blue-green algal bloom composed of *Microcystis aeruginosa* and *Aphanizomenon flos-aquae* was noted. *Microcystis aeruginosa* dominated in the bloom of Zlatari - S1 (80%), while in the basin of Vasići - S2 aforementioned species of blue-green algae were equally represented. Going further downstream, there was a shift of these two cyanobacterial species so, in Vodozahvat - S3 *Aphanizomenon flos-aquae* was dominated (95%). Qualitative analysis of surface layer showed that in terms of diversity, in three basins, Chlorophyceae were dominated (50%, 45% and 62%). Subdominant were Dinophyceae (S1, 19%), Bacillariophyceae (S2, 27%) and Cyanophyceae and Dinophyceae (S3, 15% each). In the middle layer and near the bottom, throughout the reservoir, the share of diatoms increased, while the share of green algae decreased. In terms of diversity, diatoms were dominant, while green algae were subdominant. The fact that phytoplankton community was characterized by the Cyanophyceae - Chlorophyceae - Bacillariophyceae type indicated higher trophic levels and the accelerated process of eutrophication. In terms of the use as drinking water, a particular threat was a component of water bloom, *Microcystis aeruginosa*, which cells by disintegration release high concentration of microcystins having a detrimental effects against the human and animal health.

Key words: phytoplankton, drinking water, cyanobacterial bloom, human and animal health

Introduction

Cyanobacteria (or blue-green algae) often dominate phytoplankton communities under certain conditions such as: nutrient-enriched waters and warm temperatures (ranging from 15 to 30 °C). Morphometry of aquatic systems, low grazing rates by large zooplankton have been considered advantageous conditions for the development of those kinds of bloom as well *(Zurawell et al., 2005)*. According to the World Health Organization *(WHO, 2003)*, 50-75% of the cyanobacterial blooms have been detected to be toxic. Widely-distributed toxin-producing cyanobacteria in freshwaters include N_2 fixing genera of *Anabaena, Apnanizomenon, Nostoc*, *Cylindrospermopsis* and the non-N_2 fixing genera as *Microcystis, Planktothrix* and *Oscillatoria (Welker et al., 2004)*.

Cyanobacteria and their toxins can cause major water quality problems *(Huisman et al., 2005)* through the effect of neurotoxins, hepatotoxins, cytotoxins and skin irritants, which pose threats for humans and animals *(Oberholster et al., 2004; Wiegand and Pflugmacher, 2005; Ibelings and Chorus, 2007; Sotero-Santos et al., 2008)*.

The toxins associated with cyanobacteria are mostly intracellular in healthy blooms and only affect stock following direct ingestion of cells (either in the water or as dried mats left on the shore), or from drinking water where the death of cells has caused a considerable release of toxins into the water supply. In the latter situation it may take weeks for toxins to be degraded by naturally occurring bacteria *(Carmichael, 1994)*.

Worldwide, the most common cyanobacterial toxin is microcystin, a hepatotoxin which is produced predominantly by the genus *Microcystis*. There may be some differences between animal species in the symptoms of this type of poisoning, but typically they include a display of weakeness, lethargy, anorexia, paleness, sometimes mental derangement, and often accompanied by diarrhoea. In serious cases animals suffer general distress, muscle tremors and coma which is followed by death within a few hours to a few days. Animals, particularly cattle, which survive hepatotoxicosis may suffer from photosensitisation resulting in cows refusing to suckle their young *(Carmichael and Falconer, 1993)*.

The present study described some aspects of the structure of the phytoplankton community in Ćelije Reservoir during a brief episode of cyanobacterial bloom.

Materials and Methods

Study area: Ćelije Reservoir (43°24'25"N, 21°9'57"E) was formed on river Rasina, 30 km from the town of Kruševac, central Serbia. The reservoir includes three basins: Zlatari, Vasići and Vodozahvat (water-scoop) (see Figure 1). The main characteristics of reservoir are: area = 4 km^2, max volume = $65 \cdot 10^6$ m^3, max depth = 48 m, avg depth = 12.3 m and water retention time of about 79 days.

Properties of water and phytoplankton sampling: Sampling was carried out two times per week during September 2013, at three sites in the reservoir: S1 (in Zlatari basin, near inflow of river Rasina), S2 (in Vasići basin) and S3 (in Vodozahvat basin, near the dam) (Figure 1). At these sites, water samples were collected from the surface (0.5 m), the middle (S1 - 4 m, S2 - 9 m and S3 - 10 m) and just above the bottom (S1 - 8.5 m, S2 - 18 m and S3 - 21 m). Water temperature, pH and transparency (from Secchi Disk readings) were measured *in situ.*

Chlorophyll *a* was determined as recommended by *APHA-AWWA-WEF (1998).*

Qualitative analysis of phytoplankton was performed on samples collected with conical plankton net of 20 µm *mesh* size and samples were preserved in 4% formaldehyde solution.

Taxonomic identification was performed down to the lowest possible level using an Axiovert Zeiss microscope with 2560 maximum magnification and consulting specialized literature *(Round et al., 1990; Anagnostidis and Komárek, 1989; Hoek et al., 1995).*

For quantitative analysis, samples were collected in Van Dorn bottles at the surface level (0.5 m) and fixed in Lugol's preservative. Populations were counted under an inverted microscope (Zeiss) at a magnification of 400x. The individuals (cells, colonies, cenobia and filaments) were counted in random fields and densities were calculated as recommended by *APHA-AWWA-WEF (1998).*

Figure 1. Reservoir Ćelije. S1, S2, S3 - sampling sites

In order to identify significant differences between abiotic and biotic parameters at the sampling sites, the ANOVA one-way was employed. P-values less than 0.5 were considered to be significant.

Results and Discussion

Table 1 shows the values of the examined parameters of water at three sampling sites at three different depths. The measured temperatures of water in basins pointed to the beginning of the disruption of the summer stratification, and that the process of autumn water circulation was in the course. Warm water temperatures (about 20 °C) are considered favourable for the growth of phytoplankton *(Sotero-Santos et al., 2010)*. Recorded pH values in all basins were outside the scope of the second class of waters, or for moderately eutrophic systems (6.8 - 8.5), and thus, the maximum recorded values in the surface layers of the three basins (9.22 - 9.86) pointed to the enormous production of phytoplankton and eutrophic status of reservoir Ćelije. Measured values of transparency indicated that, according to the classification of *OECD (1982)*, the reservoir Ćelije was classified as eutrophic system. The concentrations of chlorophyll *a* in all three basins (Table 1) indicated a high degree of reservoir trophic status. According to *Chapman (1997)*, the maximum value of Chl*a* in the range of 25-75 mg/l define the eutrophic status of a lake.

Table 1. Parameters of water at three sampling sites

Parameters	S1			S2			S3		
	0.5 m	**4 m**	**8.5 m**	**0.5 m**	**9 m**	**18 m**	**0.5 m**	**10 m**	**21 m**
Water temp. (°C)	22.4	20.5	19.5	22.8	19.2	15.5	22.7	19.0	13.3
pH	9.31	8.98	8.12	9.22	8.44	7.78	9.86	8.35	7.85
Secchi (m)	0.78			1.30			0.95		
Chl *a* (µg/l)	52.75	-	-	56.53	-	-	72.94	-	-

When it comes to water temperature nad pH value, there was no statistically significant differences between the individual basins. However, due to the increased production of phytoplankton, the pH value of water in the surface layer, in the whole reservoir, was significantly higher than the value recorded near the bottom. Water transparency was significantly higher in Vasići and Vodozahvat, in relation to Zlatari, because this basin (Zlatari) is the shalowest and it is under the direct influence of tributaries. The concentration of Chl *a* was significantly highest in Vodozahvat, while there was no significant difference between Zlatari and Vasići.

In the phytoplankton of reservoir Ćelije, at the time of sampling, at the end of the summer aspect, when the production was still extremely high, the following classes of algae were recorded: Cyanophyceae, Bacillariophyceae, Dinophyceae, Euglenophyceae and Chlorophyceae. Members of all classes were represented in all basins except the class of Euglenophyceae whose presence was not noted in the basin of Vasići. The greatest diversity of phytoplankton was recorded in Zlatari (S1, 41 taxa), while it was significantly lower in the other two basins (S2 - 32 taxa, S3 - 30 taxa).

Quantitatively, in the first two basins diatoms (Bacillariophyceae) dominated; at site S1 there were 27 species and varieties, with the representation index of 63.55%, and at site S2 there were 19 species and varieties, with the representation index of 60.05% (Figure 2). The green algae were subdominant (S1 - 21.10%, S2 - 31.76%). In the third basin (S3), diatoms and green algae were equally represented (Figure 2).

The analysis of the phytoplankton community showed a clear vertical stratification. Thus, in the surface layer of water (in all three basins) the blue-green algal bloom composed of *Microcystis aeruginosa* and *Aphanizomenon flos-aquae* was noted (Table 2). Since the abundance of the community where there is a blooming phenomenon of the water does not quantify, based on the other methods of assessment (graphics and subjective), it was found that the share of these species in the total number of phytoplankton was a hundred percent. *Microcystis aeruginosa* dominated in the bloom of Zlatari (80%), while in the basin of Vasići aforementioned species of blue-green algae were equally represented. Going further downstream, there was a shift of these two cyanobacterial species so, in Vodozahvat *Aphanizomenon flos-aquae* was dominated (95%) (Table 2).

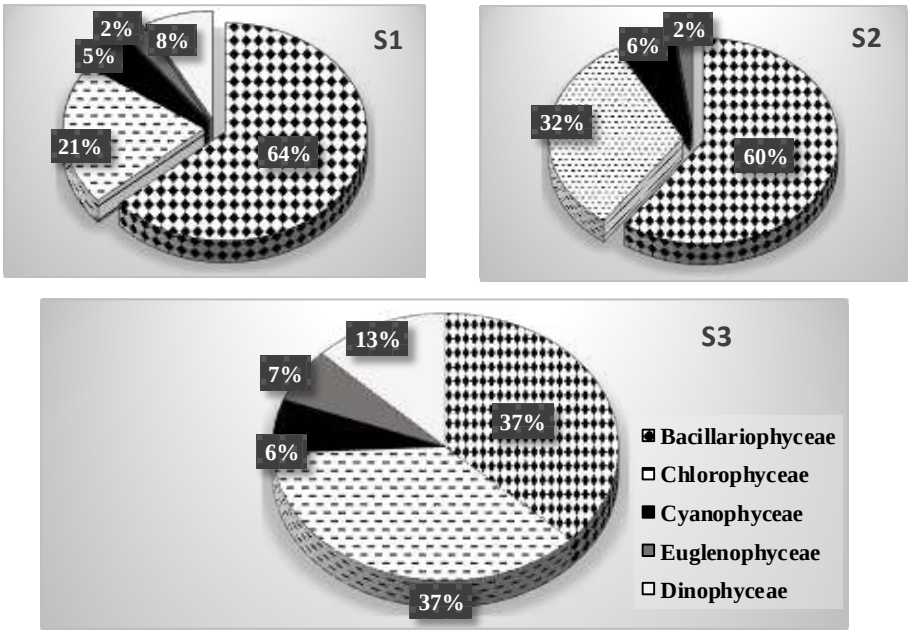

Figure 2. Percentage share of certain classes of phytoplankton at three sampling sites

The presence of the species from the other classes of algae was individually, except green algae *Pediastrum simplex* which was present in a large numbers in the whole reservoir. However, qualitative analysis of surface layer showed that in terms of diversity, in three basins Chlorophyceae were dominated (50%, 45% and 62%) (Table 3). Subdominant were Dinophyceae (S1, 19%), Bacillariophyceae (S2, 27%) and Cyanophyceae and Dinophyceae (S3, 15% each). Bacillariophyceae were not found in the surface layer of Vodozahvat.

In the middle layer (4 m and 9 m), at sites S1 and S2, green algae (*Pediastrum simplex*) were dominated, while diatoms were subdominant. At the site S3, in the middle layer (10 m), Cyanophyceae (*Aphanizomenon flos-aquae*) were dominant, while Chlorophyceae were subdominant (Table 2). In the middle layer, in relation to the surface, there was an increase in the number of taxa in basins, ie. throughout the reservoir. In terms of diversity, Bacillariophyceae were dominated (their number of taxa increased in relation to the surface), while the Chlorophyceae were subdominant (their number of taxa was reduced or remained the same in relation to the surface) (Table 3).

In the samples which were taken near the bottom, the density of the phytoplankton community was significantly higher ($p<0.05$) in Zlatari, compared to other basins (Table 2). Throughout the reservoir, the share of diatoms increased, while the share of green algae decreased. In terms of diversity, diatoms were

dominant, while green algae were subdominant. The share of other groups was significantly lower.

Table 2. The density of phytoplankton in the reservoir

Sampling site	Depth (m)	Species	Density (ind/ml)*	%
S1	0.5	*Microcystis aeruginosa/ Aphanizomenon flos-aquae*	Bloom	100 (80/20)
	4	Bacillariophyceae Chlorophyceae Cyanophyceae	14 60 2	18.42 78.95 2.63
	8.5	Bacillariophyceae Chlorophyceae Cyanophyceae	150 31 2	81.97 16.94 1.09
S2	0.5	*Microcystis aeruginosa/ Aphanizomenon flos-aquae*	Bloom	100 (50/50)
	9	Bacillariophyceae sp. Chlorophyceae (*Pediastrum simplex)* Cyanophyceae Dinophyceae	10 33 5 1	20.41 67.35 10.20 2.04
	18	Bacillariophyceae Chlorophyceae	14 10	58.33 41.67
S3	0.5	*Microcystis aeruginosa/ Aphanizomenon flos-aquae*	Bloom	100 (5/95)
	10	Bacillariophyceae Chlorophyceae Euglenophyceae Cyanophyceae	6 38 1 114	3.77 23.90 0.63 71.70
	21	Bacillariophyceae Chlorophyceae Cyanophyceae	4 7 11	18.18 31.82 50.00

The composition and structure of the phytoplankton community in the reservoir as a whole and in its separate basins, as well as the presence of bioindicator species, indicating the trophic status of the reservoir. The fact that phytoplankton community was characterized by the Cyanophyceae - Chlorophyceae - Bacillariophyceae type indicated higher trophic levels and the accelerated process of eutrophication. These results are consistent with results of other authors who have examined similar reservoirs *(Gavrilović et al., 2014; Rolland et al., 2013).*

In terms of production, high trophic level was determined by the existence of cyanobacterial bloom in the surface layers of the basins, ie. reservoir as a whole. The presence of a relatively numerous population (relative to the total number) of one species from the class of Cyanophyceae - *Pediastrum simplex*, which is

indicator of eutrophic status, was striking. Representatives of the class of Dinophyceae as indicators of the moderate trophic levels (mesotrophicity) did not achieve any significant numbers. Bacillariophyceae, as indicators of moderate and lower trophic levels (oligotrophicity) also did not achieve greater abundance.

Table 3. Diversity of phytoplankton in the reservoir (number (%) of taxa)

Sampling site	Depth (m)	Taxa	No.	%
S1	0.5	Chlorophyceae	8	50.0
		Dinophyceae	3	19.0
		Bacillariophyceae	2	12.5
		Cyanophyceae	2	12.5
		Euglenophyceae	1	6.0
	4	Bacillariophyceae	13	56.5
		Chlorophyceae	6	26.1
		Cyanophyceae	2	8.7
		Dinophyceae	2	8.7
	8.5	Bacillariophyceae	22	71.0
		Chlorophyceae	6	19.3
		Cyanophyceae	2	6.5
		Dinophyceae	1	3.2
S2	0.5	Chlorophyceae	5	45.0
		Bacillariophyceae	3	27.0
		Cyanophyceae	2	18.2
		Euglenophyceae	1	4.9
		Dinophyceae	1	4.9
	9	Bacillariophyceae	10	55.6
		Chlorophyceae	5	27.3
		Cyanophyceae	2	11.4
		Dinophyceae	1	5.7
	18	Bacillariophyceae	11	61.0
		Chlorophyceae	4	22.2
		Cyanophyceae	2	11.2
		Dinophyceae	1	5.6
S3	0.5	Chlorophyceae	8	62.0
		Cyanophyceae	2	15.0
		Dinophyceae	2	15.0
		Euglenophyceae	1	8.0
	10	Bacillariophyceae	10	47.6
		Chlorophyceae	6	28.6
		Cyanophyceae	2	9.5
		Dinophyceae	2	9.5
		Euglenophyceae	1	4.8
	21	Bacillariophyceae	8	42.1
		Chlorophyceae	7	36.8
		Cyanophyceae	2	10.5
		Diunophyceae	2	10.5

With the exception of the surface layers of the water, where the production was enormous, phytoplankton of the deeper layers, in terms of summer stratification whose disruption has been started, reached abundance in all of three basins that ranged from 22 ind./ml (Vodozahvat, 21 m) up to 183 ind./ml (Zlatari, 8.5 m). According to this parameter, the deeper layers had a character of much less productive layers in relation to the surface (oligotrophicity and mesotrophicity) *(Felföldy, 1974)*.

Conclusion

Characteristics of abiotic (temperature, pH value, transparency) and biotic (Chl*a*, qualitative and quantitative composition of phytoplankton) parameters of reservoir Ćelije showed that, in the study period, the reservoir was in eutrophic status with the indications of hypereutrophicity when it comes to the level of biological productivity.

The appearance of a bloom on the surface of the entire reservoir contributed to the high level of eutrophication. In terms of the use as drinking water, a particular threat was a component of water bloom, *Microcystis aeruginosa*, which dominated in the basin of Zlatari, and was present in the basin of Vasići in a high percentage. Disintegration of cells of this cyanobacteria/blue-green algae leads to the release of high concentration of microcystins having a detrimental effects against the human and animal health.

Bearing in mind that the water bloom appeared only briefly during the late summer, and that *M. aeruginosa* was in insignificant number in Vodozahvat (water-scoop), this water can be used for drinking after expertly selected processing procedures.

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Sastav fitoplanktona izvorišta vode za piće u periodu cijanobakterijskog cvetanja

S. Ćirić, B. Milošević, Z. Ilić, Z. Spasić

Rezime

Cijanobakterije ili modrozelene alge mogu da uzrokuju velike probleme u kvalitetu vode produkujući toksine koji su opasni po zdravlje ljudi i životinja. U ovom radu ispitana je struktura fitoplanktonske zajednice u akumulaciji Ćelije i njenim pojedinačnim basenima u toku kratkog perioda cijanobakterijskog cvetanja. U površinskom sloju vode (u sva tri basena), vodeni cvet modrozelenih algi činile su vrste *Microcystis aeruginosa* i *Aphanizomenon flos-aquae*. *M. aeruginosa* je bila dominantna u basenu Zlatari (S1, 80%), dok su u basenu Vasići (S2) pomenute vrste bile podjednako zastupljene. Idući dalje, nizvodno, dolazi do smene ove dve vrste, tako da je u basenu Vodozahvat (S3) dominirala *A. flos-aquae* (95%). Kvalitativna analiza površinskog sloja vode pokazala je da su, u pogledu diverziteta, u sva tri basena, dominirale alge iz klase Chlorophyceae (50%, 45% i 62%). Subdominantne su bile Dinophyceae (S1, 19%), Bacillariophyceae (S2, 27%) i Cyanophyceae i Dinophyceae (S3, po 15%). U središnjem sloju i u sloju vode neposredno iznad dna, u celoj akumulaciji, došlo je do porasta udela silikatnih algi, dok je udeo zelenih algi opao. U pogledu diverziteta, silikatne alge su bile dominantne, dok su zelene bile subdominantne. Činjenica da je fitoplanktonska zajednica bila okarakterisana tipom Cyanophyceae-Chlorophyceae-Bacillariophyceae ukazuje na više trofične nivoe i na ubrzani proces eutrofizacije. U smislu upotrebe ove vode za piće, posebnu opasnost predstavlja vrsta *M. aeruginosa*, čije ćelije nakon raspadanja oslobađaju visoke koncentracije mikrocistina koji pogubno deluju na zdravlje ljudi i životinja.

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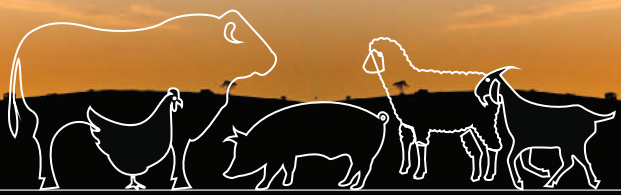

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