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SOME ASPECTS OF DNA ANALYSIS IN THE SELECTION OF SMALL RUMINANTS

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Review paper

Abstract: DNA analysis can have great practical application in the management and successful operation of the farm. The application of DNA technology is becoming a tremendous challenge for farm breeding of domestic animals. In recent years, traditional selection methods have been supplement by the results of molecular analysis of the genome. Determining the genetic distance of sheep and goat breeds had practical and multiple significance. Microsatellites are used widely in the selection, and genomic selection is becoming an increasing challenge for breeders. The development of SNP chips brings an immense advanced for rapid and comprehensive analysis of the genome, which is of great importance for the successful selection and Genomic selection in sheep concentrated on all aspects of genetic and production directions. However, it should be noted that genomic selections in sheep breeding, and especially in goat breeding, take place at a slower pace compared to cattle breeding.

Key words: selection, molecular genetics, microsatellites, small ruminants

Introduction

The traditional selection of domestic animals has based on the application of quantitative and population genetics methods. To estimate the value of the population, it needs mathematical-statistical ways with computer software required *(Caro Perovic et al., 2012a, 2012b, 2013a, 2013b, 2014).*

In sheep and goat breeding, traditionally selection methods are increasingly supplemented by modern DNA analyzes to detect genes that affected expressions of certain production traits or have located in the genome near the place responsible for a given trait (*Carillier et al., 2013, 2014; Petrović et al., 2015*).

The application of DNA analysis can have great practical application in the management and successful operation of the farm in the following way: Formation of an appropriate multilocus genotype for the identification of each animal; determining susceptibility to diseases such as Scrapie in sheep; introduction of DNA pedigree for use in long-term genetic improvement programs; resolving genealogical and ownership disputes, as well as confirming parenthood (paternity / maternity). In addition to the above, molecular genome analysis is valuable in forming a multilocus genotype database for use in forensic research and monitoring of animal products.

It is well known that the use of genetic markers has made it possible to detect the responsible genes for exhibiting significant traits or to determine their approximate location in the genome So, genetic markers are not genes that find production or other traits but show a specific place in the genome where are those genes potentially located. In addition, genes have been identified in recent years.

Microsatellite markers could use to analyze genetic diversity in sheep breeds (*Gaouar et al., 2012; Liu et al., 2014; Ebrahimi et al., 2017; Xia et al., 2021), goat (Bindu et al., 2012; Hussain et al., 2013; Seilsuth et al., 2016; Asroush et al., 2018),* and the other domestic animals (*Petrovic et al., 2018*).

This review paper aims to clarify some of the more important methods used in sheep and goat selection.

Genetic diversity of populations

In consideration of the eighth decade of the 20th century, with the development of molecular genetics methods, sheep breeding was among the first to meet modern selection procedures. Most of the known genetic markers are being used experimentally and in practice.

The use of microsatellites had a special echo in the selection of sheep and goats to characterize certain breeds and determine their genetic distance. These studies are still relevant today *(Liu et al., 2014; Zinovieva et al., 2015*).

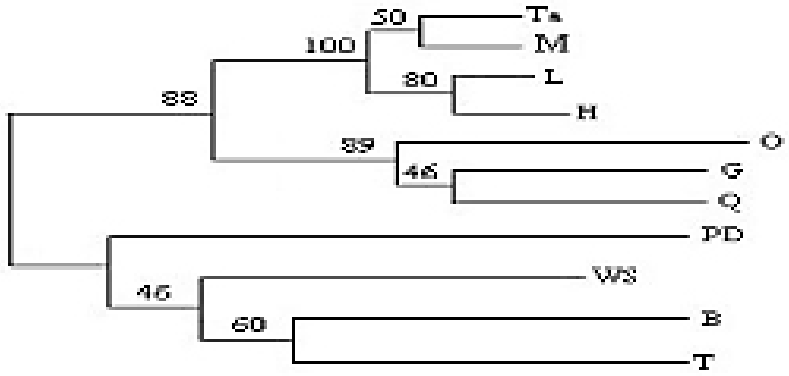

Figure 1. A neighbour-joining dendrogram of 11 mutton sheep populations based on Nei's standard genetic distances (*Liu et al., 2014*)

Table 1. Observed and expected levels of heterozygosity at microsatellite loci (*Zinovieva et al., 2015*).

Breed	F_{is}	Degree of heterozygosity		p
		observed	expected	
Grozny (GR)	0.2495	0.619±0.039	0.830±0.017	p<0.001
Stavropol (ST)	0.3520	0.567±0.031	0.881±0.012	p<0.001
Soviet Merino (SM)	0.2702	0.614±0.030	0.850±0.021	p<0.001
Edilbaevskaya (ED)	0.2776	0.595±0.032	0.853±0.023	p<0.001
Karakul (KR)	0.0627	0.724±0.035	0.808±0.036	p>0.05
Romanov (RO)	0.0591	0.707±0.029	0.779±0.031	p>0.05

Since 2010, when the international consortium for genome research of 23 sheep breeds developed the Illumina Ovine SNP50K chip, a new phase of genomic selection of sheep has begun.

In recent years, more than 50,000 SNPs have been tested to see their association with individual production traits. By the way, the Illumina SNP50K chip is a small glasses plate that has12 panels where 50,000 SNPs have for each animal. It reveals which nucleotide is present at a particular SNP site.

The obtained signals for each of the 50,000 SNP markers have transformed into the "SNP marker genotype" (AA, AB, or BB). In this way, the final result obtained in the form of a genotype for 50,000 SNP markers in each genotyped sheep.

Since 2011, the chips are also being used in goat breeding, and the Illumina SNP50K chip has been developed using the genome of 25 breeds of goats

In a relatively short period from the discovery of this method until today, thousands of SNPs have been discovered for which the exact position in the genome is known, as well as the consequence of the change in the nucleotide base.

For example, some studies have shown that sheep with heavier fleece in a particular SNP have an adenine (A) base, with animals with a lighter fleece in the same SNP having a guanine (G) base (*Petrovic and Pantelic, 2015*). The use of the SNP chip technique has begun to be applied in the selection of sheep and the determination of the genetic connection of populations (*Deniskova et al., 2019*).

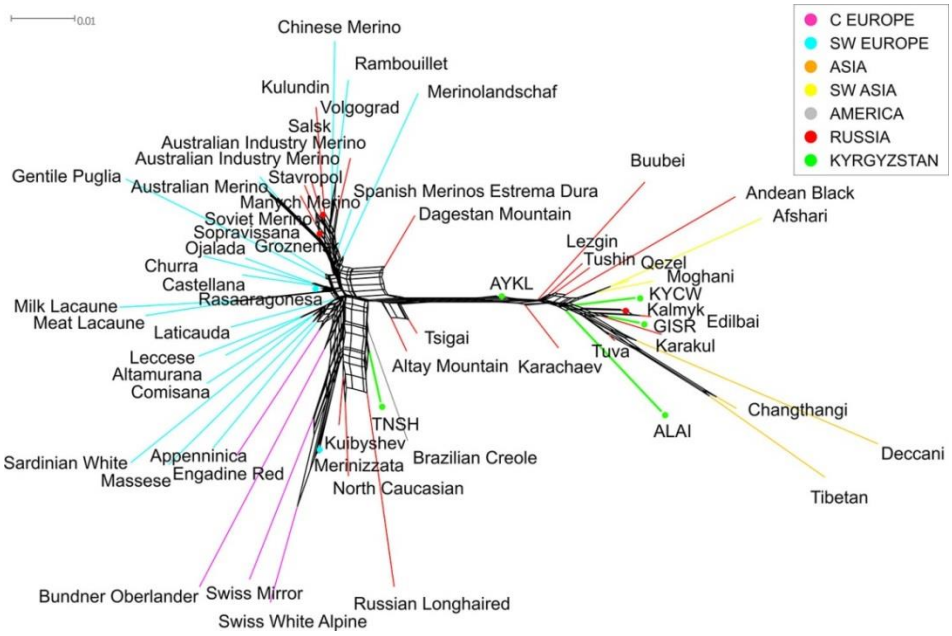

Figure 2. Characterization of the population structure sheep breeds, and to study their genetic connections using the ovinesnp50k beadchip and the Ovine Infinium HD beadchip (Illumina Inc., USA).

The application of the SNP genome detection procedure may have different applications. For example, *Kijas et al. (2009)* developed a set of SNPs distributed throughout the sheep genome. Relied on re-sequencing over 2600 genomic targets that have a known location within the virtual genome of sheep to use such a SNP set, the mentioned researchers performed genotyping. They thus determined the level of polymorphism between the examined sheep populations. The authors state that the results led to the knowledge of how sheep populations are grouped on the basis of geographical origin, whereby a modest number of SNPs can successfully identify the population substructure within individual breeds. Part of the results is shown in Figure 3.

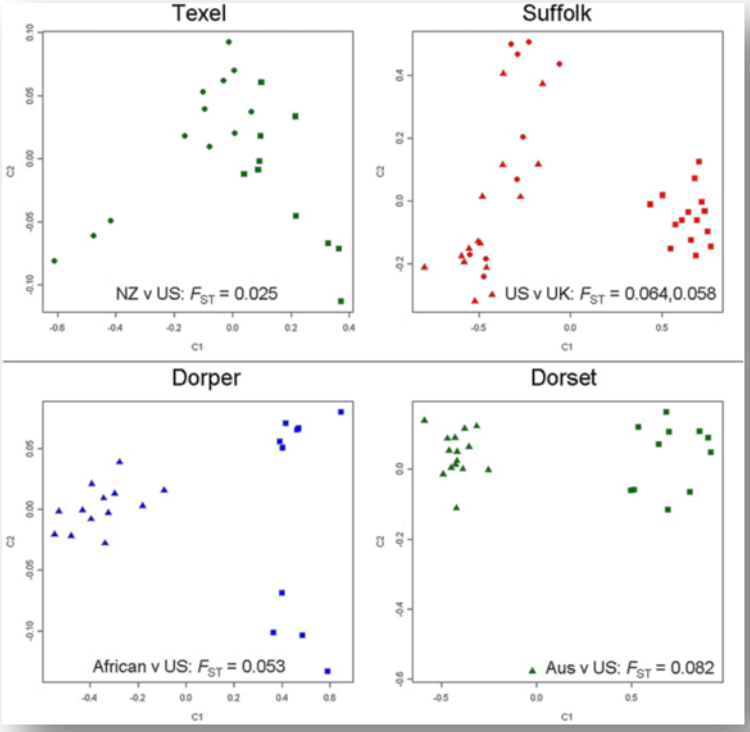

Figure 3. Multidimensional scaling shows genetic differences between geographically separated populations *(Kijas et al., 2009)*

Application of genomic selection in sheep breeding

Genomic selection application in sheep breeding is gaining momentum, and among the leading countries of such as Australia, New Zealand, Russia, China, more precisely the countries with the most developed sheep breeding. When it comes to European countries, France stands out the most, especially with the dairy breed Lacon.

Chinese researchers have discovered SNPs58995.1 (position 3858663 in contig) located in the regulatory zone of the myocyte enhancer gene; factor-2 (MEF2B) which has a large impact on meat quality (*Zhang et al., 2013*). The MEF2B gene encodes a protein from the MEF2 family. Proteins interaction from the MEF2 family with the promoter myostatin genome of sheep has a stimulating effect on the expression of myostatin, a protein that restricts muscle growth in mammals (*Du et al., 2007*). Therefore, a mutation in the MEF2B gene can affect

sheep meat production by altering myostatin production. It's been confirmed in some other studies (*Chen et al., 2015*).

Milk production and traits in the Spanish breed Churra were studied by *Garcia-Gamez et al. (2012)* and found a link to certain genes. Large numbers of regions identified associated to milk traits (*Usai et al., 2019).*

Carillier et al. (2015) state that the availability of the SNP54k chip for goats, enabled the genotyping of 825 goats for the Alpine and San goat breeds in France. In both races, genomic selection can improve annual genetic progress by reducing the length of the father-son time interval. The quality of predicting the value of an individual in this way is sensitive to the size of the reference population, which can affect the accuracy of genomic indicators.

Genomic selection in sheep concentrates on all genetic aspects and production directions. However, it should be noted that genomic selections in sheep breeding, and especially in goat breeding, take place at a slower pace compared to cattle breeding. However, this is only conditionally said because each of these branches of animal husbandry has its own significance, goals and selection requirements depending on different natural and social influences.

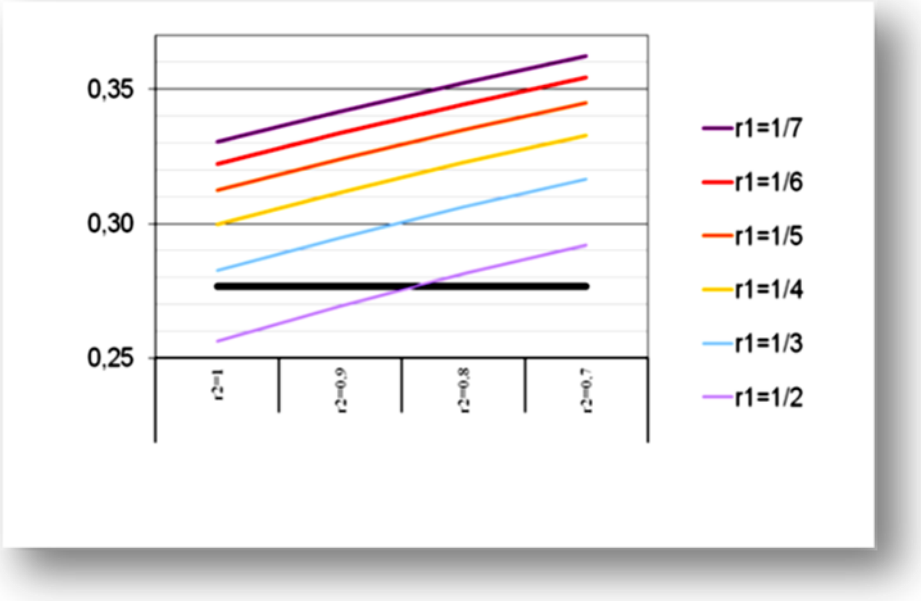

Figure 4. Annual genetic progress (in genetic standard deviation) according to r1 (genomic selection) and r2 (selection after progeny test) for Lacon sheep breed (*Buisson et al., 2013*)

Conclusion

The use of molecular DNA analysis techniques in sheep and goats is of great theoretical and practical importance. Knowledge of the genetic distance of populations is necessary in order to avoid homozygosity but also in crossbreeding programs for sheep breeds. Microsatellites, in this sense, played an important role in selection.

The results of the obtained SNP genotypes could apply to determine the history and diversity of sheep and goat populations within our country and the world. It is also possible to determine the origin of individuals, genetic variations that are associated with some diseases, with horns, some characteristics of carcasses, etc.

Finally, we can summarize how the genomic selection of sheep and goats had the following advantages: More accurate prediction of genetic value for the desired breeding goal; in traits that are usually difficult to improve; for properties that are difficult or expensive to measure; for properties that cannot be measured early; in traits with low heritability, for example: yield and meat quality traits, lifelong wool production, reproductive rate, parasite resistance.

Neki aspekti DNK analize u selekciji malih preživara

Violeta Caro Petrović, Dragana Ružić Muslić, Nevena Maksimović, Bogdan Cekić, Ivan Ćosić, Marina I. Selionova, Milan Petrović

Rezime

DNK analiza može imati veliku praktičnu primenu u upravljanju i uspešnom poslovanju farme. Primena DNK tehnologije postaje veliki izazov za selekciju domaćih životinja, pa se poslednjih godina tradicionalne metode selekcije dopunjuju su rezultatima molekularne analize genoma. Određivanje genetske distance rasa ovaca i koza ima višestruki praktični značaj. Mikrosateliti se široko koriste u selekciji, a genomska selekcija postaje sve veći izazov za odgajivače. Razvoj SNP čipova donosi veliki napredak u brzoj i sveobuhvatnoj analizi genoma, što je od velikog značaja za bržu selekciju ovaca i koza. Genomska selekcija je skoncentrisana na sve genetske aspekte i pravce proizvodnje. Posebno na osobine koje se teško mere ili se ne mogu izmeriti u ranom uzrastu jedinke. Međutim, treba napomenuti da se genomska selekcija u ovčarstvu, a posebno u kozarstvu, odvija sporijim tempom u odnosu na govedarstvo.

Ključne reči: selekcija, molekularna genetika, mikrosateliti, mali preživari

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FARMERS' ECONOMIC INTEREST IN *DERMANYSSUS GALLINAE* CONTROL

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Review paper

Abstract: Poultry red mite or *Dermanyssus gallinae* (De Geer, 1778) is the most significant poultry ectoparasite with regards to health and economy. It is a widely accepted opinion that *D. gallinae* can only be suppressed, with the current annual expenditure of 60 eurocents per layer. However, research indicates that *D. gallinae* can be controlled in other ways and eradicated from the production facilities and farms, and subsequent reinfestation can be prevented by implementing biosafety measures. This provides a long-term or permanent effect of *D. gallinae* control. From the aspect of economy, this means that after decades of increasing expenditures, farmers can first decrease, and then completely eliminate expenditures incurred by *D. gallinae*. Therefore, economic calculations should be based on an expert and comprehensive approach, which should itself be based on rational control, preventive veterinary medicine, i.e. *D. gallinae* control program. This would result in long-term savings. In 10 years' time, 0.5 million euros would be saved per 100.000 layers. There are an estimated 4 billion infested layers worldwide.

Key words: *Dermanyssus gallinae*, control, economic interest

Introduction

Poultry production is the field of animal husbandry which provides the largest portion of animal source foods for human consumption. Last decades have been marked by an intensive increase in egg production. Over the period from 1970 to 2007, the production of table eggs tripled and rose from 20 million to 60 million tons. According to FAO, the number of layers reached 4.93 billion in 2009 *(FAO, 2010)*.

Poultry red mite is the most significant poultry ectoparasite with regards to

health and economy (*Nordenfors, 2000*). Control of red poultry mites in the current situation, not paying enough attention to the choice of acaricidal products for control and methods, i.e. rational pharmacotherapy (suppression), professional application and the principle of preventive veterinary medicine. Therefore, simultaneously with the increase in egg production, the problem of prevalence and harmful effect of poultry red mite has also increased, together with the damage caused by inefficient, partly efficient, or illegal control (*Giangaspero et al., 2011, 2017; Marangi et al., 2012*)

Poultry red mite

Poultry red mite, *Dermanyssus gallinae* (De Geer, 1778) is an invasive arthropod, successfully adapted to the conditions of modern poultry production (figure 1). Over 80% of commercial layer flocks, as well as parent and breeding flocks are affected by the invasion of this ectoparasite (*Sparagano et al., 2009*). Its small size (about 1 mm), mobility, ability to feed on a large number of species of birds and mammals, resistance to temperature conditions and starvation (*Pavlićević et al., 2007b*), extreme adaptability and development of resistance, large numbers and high reproductive potential, hidden way of life and night activity (*Simić and Živković, 1958*) are the traits of this parasite which enable its invasiveness. Poultry red mite is a problem of the flock, but also of the environment, thus jeopardising not just current, but also future flocks (*Pavlićević et al., 2018b*).

Figure 1. Microscopic image of *D. gallinae*. The mites get their red colour from freshly consumed blood *(Photo: Zeković Miljan & Pavlićević Aleksandar)*

In addition to biological traits of the parasites, the basis of this health and economic problem is a long-term wrong approach to *D. gallinae* control, which has been additionally exacerbated by the new changes (EU 1999/74/EC) in the technological conditions of housing layers (*Pavlićević et al., 2016a, 2016c, 2019; Flochlay et al., 2017*).

Figure 2. *D. gallinae* invasion in the clinical conditions of commercial egg production, cage system. Hundreds of thousands of these parasites can parasitise on one laying hen
(Photo: Pavlićević Aleksandar)

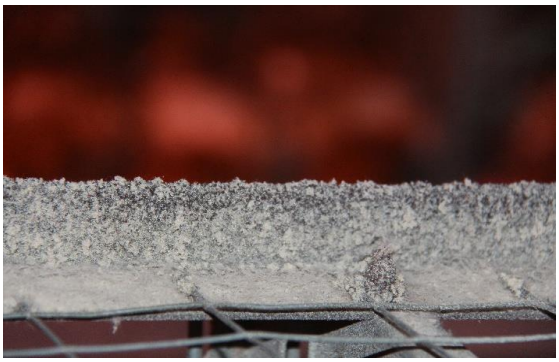

Figure 3. *D. gallinae* invasion in the clinical conditions, a detail of the upper, inner edge of the cage with massive clusters *(Photo: Pavlićević Aleksandar)*

Harmful effects

The harm caused by *D. gallinae* can be direct and indirect.Direct harm is caused by immediate parasitic action of *D. gallinae*: crawling on the body, stinging and bloodsucking. This results in poultry being afflicted by stress, anaemia due to blood loss, deteriorated general health state and immune status, aggravation and transmission of infectious diseases. Clinical manifestations in the flock are nervousness, pronounced health problems, increased mortality rates, reduced egg quality, and increased feed consumption (*Emous 2005, 2017; Flochlay et al., 2017*).

D. gallinae is a zoonosis and occupational disease (*Cafiero et al., 2019*). It also afflicts people, causing nervousness, itching, and changes on the skin. These problems can result in workers leaving their jobs or asking for compensation due to aggravated working conditions.

Further, indirect harm caused by *D. gallinae* occurs due to transmission and incorrect approach.

1. A young flock can be invaded in the rearing facility, and it can further transmit the infestation to a previously uninfested production facility, thus

causing further damage. The disease caused by *D. gallinae* (*Dermanyssosis*) is a hidden flaw, but it can be detected if properly looked for. The condition necessary to avoid the damage is a correct forensic assessment and definition of legal relations (*Pavlićević et al., 2003, 2018c*).

2. Used cages and equipment are some of the key vectors of *D. gallinae* in intensive poultry production. When purchasing used cages and equipment, it is necessary to pay attention to possible invasion. Invaded cages and equipment should cost less, because they will incur unplanned sanitation expenditures for farmers (*Pavlićević et al., 2016b, 2018c*).
3. Transport cages for poultry transmit *D. gallinae* and thus cause damage (*Pavlićević and Pavlović 2016b*).
4. Incorrect work organisation on farms enables the transmission of invasion within the farm, from one house to another.
5. Incorrect choice of products and methods of control and/or their unprofessional application cannot provide efficient control of *D. gallinae* invasion, and it can cause damage.
6. Untimely *D. gallinae* control increases the damage by maximising direct harm, as well as making control more expensive, usually through higher consumption and lower efficacy of products. The production in highly infested flocks (++++) is not cost-effective.
7. Uncritical control is reflected in the application of illegal acaricides or inadequate application of registered products, which is harmful to human health, poultry and the environment.
8. The presence of *D. gallinae* on table eggs causes customers' disgust and aversion.
9. Highly infested flocks can result in abattoirs' refusal to accept the flock after the production period is finished.

Calculation

Expenditures incurred by the damage are added to the cost of products and implementation of control measures and they represent the farmers' total economic loss. In some cases, these expenditures can include the cost of preparation and sanitation of the consequences of application, such as the eggs which must be safely disposed of due to the withdrawal period (harmful chemical residue in eggs). Farmers' true economic loss is visible after one year, which is the duration of the production period, or over a longer period.

Farmers' economic loss is caused by the increased parasitic prevalence, intensity and extensity of the invasion, difficulty level of *D. gallinae* control, and cost of products and methods. Estimated cost per hen in the period from 2005 to

2017 increased by 40%, and it is 231 million euros annually for the whole of Europe. Annual expenditure caused by *D. gallinae* per hen is 60 euro cents, 15 of which are spent on the control and 45 on damage (1:3) (*Emous, 2005, 2017*).

Less successful, and especially unsuccessful control includes both types of expenditures. The less successful *D. gallina*e control measures are, the bigger total expenditure is for farmers. However, expenditures caused by *D. gallinae* and its control do not have to nor ought to occur simultaneously. Successful *D. gallinae* control implies only control expenditures for farmers, and, in time, even those are eliminated. For example, in 10 years' time, over 0.5 million euros would be saved per 100.000 layers (capacity of a medium size farm). If we apply the infestation rate to the number of layers (*FAO, 2007*), we get the figure of about 4 billion layers infested with *D. gallinae* worldwide. This is an approximate figure, since both the numbers of layers and infested flocks have risen in the meantime. For example, reports for Germany, the Netherlands, and Belgium put *D. gallinae* infestation in layer flocks at 94% (*Mul et al., 2016*).

Current control

Current *D. gallinae* control offers a large selection of products. In the purpose of clarity, we have selected just two most important groups of products.

Since the beginning of modern intensive poultry production, *D. gallinae* control has predominantly been based on acaricides, synthetic neurotoxic compounds. Its purpose is *D. gallinae* suppression and its effects last for several months, or in some cases for over six months (*Pavlićević, 2005; Pavlićević et al., 2016, 2018d*).

Over the past 10 years, with the development of SiO_2 formulations, a technology which can compete with acaricides has been developed for the first time. However, the progress achieved with SiO_2 has not been properly utilised, but has also been employed just in the purpose of *D. gallinae* suppression.

No developmental steps taken so far indicate any future change in the widely accepted approach to *D. gallinae* control. The control program for *D. gallinae* has been developed in contrast to the predominant approach. However, for over 20 years, it has remained marginalised and without any significant impact on the mainstream red mite control in the poultry industry.

Program

The problem of *D. gallinae* control can be solved and it does not have to exist in the poultry industry. The solution is a program, a comprehensive approach which would be based on preventive veterinary medicine and rational pharmacotherapy (control). The primary goal of the program in intensive poultry

production is to prevent *D. gallinae* infestation in uninfested poultry houses, i.e. farms. Safety risks need to be excluded in infested houses, rational control needs to be introduced, and then efficacy and cost-effectiveness will be increased. After the necessary conditions have been met, *D. gallinae* is eradicated from the production facilities on the farm, and biosafety measures are introduced (*Pavlićević et al., 2018a, 2018b*).

For example, if a highly effective suppression of *D. gallinae* is achieved by two treatments (during housing preparation, before the population) with P 547/17 (*project ID 1115*), in the partial absence of adequate conditions, mites will appear in small numbers only in the final three months. A small mite infestation (from + to ++) has no significant (measurable) health impact and it does not cause economic loss. If there are adequate conditions (hygienic conditions and housing downtime), the procedure of housing preparation with P 547/17 technology results in *D. gallinae* eradication from production facilities. In this case, the flock is not exposed even to minimal *D. gallina*e presence, i.e. its harmful effect, and therefore these harmful consequences do not exist anymore. If there is continued implementation of biosafety measures, the expenditures caused by harmful effect or further control are excluded in the future.

During its development, the program has relied on the current, available products and methods. Initially, it was based on acaricides. However, contrary to the widely accepted method of control (which required more frequent use of acaricides), a correct acaricide application in the poultry house (eradication and introduction of biosafety measures) eliminates the need for further acaricide use (*Pavlićević et al., 2016*).

The first practically applicable distancing from acaricide control was enabled by SiO_2-based formulations. By exploring the possibilities of mechanical control, an original program was developed, based on the combination of powdered and liquid forms. Eradication was possible again, this time based on a mechanical method. However, an expensive and complex technological procedure, highly demanding regarding the necessary conditions, hindered its wider implementation.

Main disadvantages of SiO_2-based product application have been successfully overcome first by developing a specialised formulation based on inert oils (P 547/17, Pulcap), and then by developing an original technology of its application (Project ID 1115). The new formulation and technology has been tested in laboratory (*Pavlićević et al., 2017a, 2017c*) and clinical conditions. In this way, we have eliminated any safety risks and devised a more functional and efficient and less complex application procedure which requires fewer conditions. There is no possibility for *D. gallinae* to develop resistance to P 547/17 or to significantly adapt its behaviour. Therefore, the current program will not lose its efficacy over time. Its results are permanent. In comparison to other programs and methods for poultry red mite control, we have concluded that P 547/17 formulation and application technology is an example of rational *D. gallinae* control (*Pavlićević et*

al., 2017b, 2019b). Moreover, it provides all the conditions necessary to completely exclude the application of neurotoxic synthetic compounds from poultry meat and egg production. P 547/17 formulation and application technology has its requirements: professional application, hygienic conditions, housing downtime. Furthermore, despite its undisputed quality, it has certain disadvantages, thus leaving more space to further improve this type of control. This program is permanently open for all new contributions to *D. gallinae* control, which would help it to function better and more adequately respond to various practical challenges of modern poultry industry.

A systematic approach to the implementation of *D. gallinae* control would be an adequate step towards intensive vertical and horizontal integration of poultry industry. Systematic program implementation would additionally contribute to functional and rational product application, protection of human and animal health and environment, and improved control of diseases transmitted by *D. gallinae*; improving the flock's general health status and increasing production results would result in farmers' economic gain.

The situation for *D. gallinae* control in extensive poultry production is different from the one in the intensive production, and consequently, the approach of the program is different. In extensive poultry production, there is an open system, contact with other domestic and wild animals, large area per layer, and complex environment. It is advised to correctly build and set up the perches and nests, together with the barriers which successfully divide them from the rest of the environment. In this way, farmers can control *D. gallinae* problem easily and without significant expenses (P 2017/ 0762).

The necessary conditions have been met to first stop the unfavourable trend in *D. gallinae* control, then mitigate the problem, and eventually eliminate it completely. All this cannot be achieved immediately, since the procedure is technologically demanding and complex.

Veterinary medicine

Insufficient efficacy of veterinary medicine in *D. gallinae* control effected the size and extent of economic loss suffered by farmers. Veterinary medicine could have contributed more to the mitigation and prevention of loss caused by *D. gallinae* in the following ways:

1. The primary role of veterinary medicine should have been to provide timely and correct information to farmers. In this way, the disease could have been stopped in its initial phase, and most farms would have been protected by biosafety measures, while the rest would have been easily treated. Well-informed farmers would have taken an active role in the solution of the problem, otherwise they make wrong decisions and become

a part of the problem (*Pavlićević et al., 2016*);

2. Defining eradication as the objective of the control in intensive poultry production is the premise for a real solution. The generally accepted opinion in veterinary medicine that *D. gallinae* can only be suppressed puts the farmers in a hopeless position of constant, increasing expenditures (*Pavlićević et al., 2018, 2018b*);
3. Improved detection and standardised laboratory and clinical testing of efficacy of products and methods for *D. gallinae* control (*Pavlićević et al., 2007a, 2017b, 2019c*);
4. Warning about technological flaws and negative effects of complex cages and equipment, which would contribute to the improvement of conditions for *D. gallinae* control (*Pavlićević et al., 2016a*);
5. Timely utilisation of the legislation change in the EU regarding cages and equipment (EU 1999/74/EC) could have easily eliminated the problem. However, the omission to do so had the opposite effect and actually contributed to the spread of the disease (*Pavlićević and Pavlović 2016a*);
6. Insisting on rational control, advising the farmers about the optimal choice of current products and methods, based on verified data;
7. Ensuring professional application of products and methods, which is crucial for efficient control;
8. Insisting on preventive veterinary medicine and maximising the efficacy of control relative to the cost;
9. Regular tests of resistance and timely elimination of unjustified use of acaricides which have already caused resistance (*Pavlićević et al., 2016*);
10. Promoting integrated health care, especially with regards to the control of infectious diseases transmitted by *D. gallinae* , which would additionally improve the general health status of poultry and contribute to the general welfare and cost-effectiveness of poultry production (*Pavlićević et al., 2017b*);
11. Improving the efficacy of *D. gallinae* control and residue monitoring and minimise or completely exclude the toxicological risk caused by uncritical control (*Pavlićević et al., 2005, 2018c*);
12. Introduction of the control program would cover all the above said requirements (*Pavlićević et al., 2018a, 2018b, 2019d*).

We are facing an open question – to what extent does veterinary medicine fulfil its role in *D. gallinae* control? Farmers' economic interest is currently not in accordance with the generally accepted opinion in veterinary medicine regarding *D. gallinae* control. The future will provide the answer to the question to what extent it is possible to critically review and improve the above-mentioned positions of veterinary medicine in accordance with the basic medical principles and in the interest of general welfare and economic interest of farmers.

Conclusion

The economic interest of poultry producers can be significantly improved. Farmers' expenditures incurred by *D. gallinae* can be reduced (time necessary to meet the conditions), and subsequently completely eliminated. Improving farmers' economic interest from the aspect of *D. gallinae* control is in correlation with the general welfare (interest). The requirements necessary in order to achieve the said interest depend on the role of veterinary medicine, which should reconsider the current procedure of *D. gallinae* control and introduce the principles of rational control and preventive veterinary medicine, i.e. the control program.

Ekonomski interes farmera u kontroli *Dermanyssus gallinae*

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Rezime

Tekut ili crvena kokošija grinja Dermanyssus gallinae (*De Geer, 1778*) je zdravstveno i ekonomski najznačajniji spoljašnji parazit u živinarstvu. Opšte je prihvaćeno mišljenje, da je *D. gallinae* moguće samo suzbijati, i da je pri tome aktuelni godišnji trošak po nosilji 60 eruo centi. Međutim, istraživanja ukazuju na to da postoje i druge mogućnosti kontrole *D. gallinae,* te da je iskorenjavanje (eradikacija iz proizvodnih objekata i farmi) moguće, a zatim, i da je moguće sprečiti njihovo naknadno unošenje (reinfestaciju) biosigurnosnim merama. Time se omogućava dugotrajan ili trajan efekat kontrole *D. gallinae*. Sa ekonomskog aspekta, to znači da nasuprot višedecenijske tendecije povećavanja troškova, farmeri mogu smanjiti, a zatim potpuno isključiti troškove koje im stvara *D. gallinae.* Prema tome, ekonomski proračun svoje uporište bi trebao da temelji na stručnoj osnovi i sveobuhvatnosti, a stručna osnova bi trebalo da bude zasnovana na racionalnoj kontroli, preventivnoj veterinarskoj medicini, odnosno programskom prilazu kontrole *D. gallinae.* Na ovaj način stvorile bi se dugoročne uštede. Za deset godina, za svakih 100.000 nosilja ušteda je preko 0,5 miliona eura. Na svetu se procenjuje da je infestirano oko 4 milijardi nosilja.

Ključne reči: *Dermanyssus gallinae*, kontrola, ekonomski interes

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INFLUENCE OF PREBIOTICS IN PIGS NUTRITION ON BODY WEIGHT AND CONTENT OF *Escherichia coli* IN FECES

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Abstract: The aim of this study was to determine the effect of prebiotics in the diet of piglets after weaning on the increase in the average weight of experimental animals between female (♀) and male (♂) piglets, dynamically by weeks of experiment, as well as on the presence of *Escherichia coli* in faeces. The experiment included piglets at weaning, aged about 26 days. The F1 generation was used, which was created by crossing Landrace sows and Pietren boars. The piglets were fed for 56 days (8 weeks). Four groups were formed, each group was composed of 10 piglets (5 females and 5 males) with different concentrations of prebiotics and controls, in two replicates, a total of 100 experimental animals. Feed mixtures consisting of the same nutrients were used in the diet. Control groups (OA and OB) were fed with ordinary feed mixture, without the addition of additives, groups (IA and IB) with the addition of Bio-Mos prebiotics, in a concentration of 0.1%, groups (II-A and II- B) with 0.2%, groups (III-A and III-B) with 0.3% and groups (IV-A and IV-B) were fed with the addition of prebiotics in a concentration of 0.4%. It was concluded that the differences observed in the average weight between ♀ and ♂ piglets in any measurement period were not statistically significant. However, the best results in terms of average weight were given by the highest used concentrations of prebiotics in food, 0.4% and 0.3%. The preparation had a positive effect in terms of reducing the number of *Escherichia coli* bacteria in the digestive tract of piglets in all treatment groups by 42 to 320 times.

Key words: pig, prebiotic, mass, *Escherichia coli*

Introduction

The European Union has completely banned the use of antibiotics as growth promoters since January 2006. Thus, the goal of many studies has become

alternative strategies for modulating the gastrointestinal environment in piglets. One of the alternative solutions is the use of prebiotics. Prebiotics have been defined by numerous authors, including (*Manning and Gibson, 2004; Awati and Moughan, 2006*) as indigestible food ingredients that act by selectively stimulating the growth and activity of non-pathogenic bacteria and have a beneficial effect on the health and production results of hosts. Fructo-oligosaccharides, inulin and mannan-oligosaccharides are some of the defined prebiotic additives intended for animal feed (*Pourabedin and Zhao, 2015*). Bio-Mos (*Alltech Inc®, USA*) is a product obtained by extraction of mannan-oligosaccharides from the outer part of the cell wall of the yeast *Saccharomices cerevisiae var. boulardii*. The effects of this preparation are as follows: it blocks the action of pathogenic bacteria by binding to them, strengthens the immune system by stimulating the synthesis of antibodies, stimulates the growth of beneficial bacteria in the intestines and thus improves conversion and production results. From previous research, it can be concluded that the best effect of Bio-Mos additive is manifested immediately after irrigation. *Radulović et al. (2014)* concluded that the use of prebiotics excludes negative effects, such as the appearance of residues, resistance and allergies, which were previously detected when using antibiotics in the diet. *Miguel et al. (2004)* conclude that its effect is most beneficial for piglets with slow growth (less than 180 g / day) during the first two weeks after weaning. Prebiotics play a significant role in preventing diarrhea during this period. Edema is a disease that most commonly affects piglets after weaning, and is caused by haemolytic strains of the bacterium *Escherichia coli*. *Priebe et al. (2002)* observed that a prebiotic can reduce the number of potentially pathogenic organisms such as bacteria from the genus *Clostridium*, from the family *Enterobacteriaceae* (genera *Escherichia coli, Enterobacter, Shigella* and *Salmonella*). The inclusion of prebiotics in the diet of piglets leads to an increase in the number of *Lactobacillus* and a decrease in the number of *Escherichia coli* in the ileum and colon of piglets, found *Deng et al., (2007)*. Results similar to the previous ones were presented by *Liu et al. (2008)* in which they state that the addition of prebiotics to the diet of weaned piglets successfully increases the number of *Lactobacillus* bacteria and reduces the number of *Escherichia coli* in faecal samples on days 14 and 21 after weaning. They also concluded that adding prebiotics to the diet of weaned piglets improves production parameters by increasing digestibility, reducing the frequency of diarrhea and improving intestinal morphology. The results of two experiments performed to examine the effect of mannan-oligosaccharides on the production results of broilers indicate that the use of Bio-Mos in the amount of 0.1 and 0.2% results in a statistically significantly higher body weight of broilers. In the first experiment, this increase was 4.8, and in the second 5.5% *(Newman, 1999)*.

The aim of this study was to determine whether Bio-Mos prebiotic affects the growth dynamics of the average weight of experimental animals, by weeks of the experiment, as well as the presence of *Escherichia coli* in the faeces. This

research should enable adequate formulation of meals with the addition of Bio-Mos prebiotics for piglets after weaning, in order to make the most adequate use of all genetic predispositions and enable more economical production of pork.

Material and Methods

The experiment included piglets of F1 generation (landras x pietren), aged about 26 days, uniform body weight, fitness and health status. Piglets were treated with a dose of vitamin AD3E, a vaccine against swine fever, and male piglets aged 12 days were castrated. Piglets were measured individually, initially when forming groups, and then every seven days. The piglets were fed for 56 days (eight weeks). Each group consisted of 10 piglets, five females and five males. Four treatment groups were formed with different concentrations of prebiotics: I with 0.1%, II with 0.2%, III with 0.3% and IV with 0.4% and control (O) without prebiotics, in two replicates (A and B groups), which means that 10 groups of piglets were formed, a total of 100 experimental animals. Feed mixtures consisting of the same nutrients were used in the diet. During the experiment, three types of mixture were used: pre-starter, starter and grower. For the treatment groups, the prebiotic Bio-Moss was used as a supplement, which was included in the diet from the second week of the experiment. The piglets were fed four times during 24 hours. The food was distributed manually and water was available ad libitum.

Faecal samples were taken on three occasions, days 34, 45, and 55 of the experiment. Collective samples were taken from each group, which were then marked and sent for analysis to the Veterinary Institute "Dr Vaso Butozan" in Banja Luka. The test method used is called isolation and identification of *Escherichia coli*. The reference for this method is: "Colour Atlas and Textbook of Diagnostic Microbiology, 5th Edition, 1997" and "Manual for laboratory diagnostics-Standardization of diagnostic methods for bacterial, viral and parasitic diseases of animals whose control is prescribed by law, Belgrade, 1984". The reference culture of *Escherichia coli* was used. Method description: Cultural examination, then the material was seeded on two nutrient media (endo agar, Mc Conkey agar). The seeded media were incubated for 24-48 hours at 37°C. On endo agar the colonies of *Escherichia coli* are red with a metallic sheen, on Mc Conkey agar the colonies are pink. During the experiment, the dynamics of the average weekly increase in the mass of experimental animals, by weeks of the experiment and the presence of the bacterium *Escherichia coli* in the faeces were monitored.
Statistical analysis of the study was performed on experimental animals of both analysed groups, A and B, so that the sample of each treatment group for both female and male piglets was represented by 10 experimental animals (n = 10 biometric observation units). The obtained experimental results are presented in the tables as average values with corresponding measures of variability, by weeks of

measurement, especially for female (♀) and male (♂) piglets. The tables show the percentage difference in achieved masses between male and female piglets with a t-test of the significance of this difference.

Results and Discussion

In accordance with the set goal and the obtained experimental results in this research, the analysis and discussion are the results of the effect of applied diet treatments on the increase of average weight of experimental animals, dynamic and analysis results of *Escherichia coli* in faeces, as a consequence of applied diet treatments.

The effect of applied food treatments on the increase in the average weight of experimental animals, dynamically

Raising piglets largely depends on the diet, which directly affects growth. This period represents the most critical phase of production. Problems that occur due to the unaccustomedness of piglets to the consumption of concentrated nutrients, insufficiently developed digestive tract for digestion as well as due to insufficient secretion of hydrochloric acid in the stomach of piglets. Losses are greatest just after the weaning. This is the period when diarrhea, indigestion and other health problems occur and all together lead to losses in the body weight of piglets. Weaning of piglets with the level of stress reduced to a minimum and the transition to the next production phase, can be partially solved by adequately balanced meals with the use of additives, which are recommended in this phase of production. Prebiotics are considered a good solution, since many studies show that they have positive effects on the growth of animals, as well as on reducing diarrhea and indigestion, because they act on bacteria, regular inhabitants of the gastrointestinal tract. The initial body weight of piglets, before the introduction to the experiment, as well as the dynamics of the increase in the average weight of experimental animals are presented in this part of the paper. The dynamics of the average weekly increase in the weight of piglets in the control group is given in Table 1.

Table 1. Dynamics of average weekly weight gain of piglets in the control group

Terms	♀ $\bar{X} \pm S_{\bar{X}}$	*Vk*	♂ $\bar{X} \pm S_{\bar{X}}$	*Vk*	% $\Delta\bar{X}$	t_{exp}:
Initial weight	7164.5 ± 72.6	3.20	6981.5 ± 104.9	4.75	2.69	1.434 *ns*
7	7416.0 ± 230.5	9.28	7190.0 ± 95.3	4.18	3.14	0.906 *ns*
14	8141.0 ± 281.8	10.95	8249.5 ± 115.1	4.41	1.33	0.356 *ns*
21	9476.0 ± 385.6	12.87	9834.5 ± 169.8	5.46	3.78	0.851 *ns*
28	11600.0 ± 534.9	14.58	12080.0 ± 256.9	6.73	4.13	0.809 *ns*
35	12945.0 ± 652.7	15.94	12915.0 ± 519.5	12.72	0.23	0.036 *ns*
42	15020.0 ± 737.3	15.52	15265.0 ± 684.9	14.19	1.63	0.243 *ns*
49	17500.0 ± 1005.2	18.16	17723.0 ± 1017.9	18.16	1.27	0.156 *ns*
56	19945.0 ± 1162.4	18.43	20235.0 ± 1146.3	17.91	1.45	0.178 *ns*

Insight into Table 1 shows that the difference in average weight between male and female piglets in terms of measurements differs from 0.23% (day 35) to 4.13% (day 28). The significance of the difference determined by the t-test between male and female piglets of the control group shows that it is not statistically significant at any time. For the final analysis of the effect of the applied feeding models, we can state that the piglets in the control group, regardless of sex, achieved an average weight of 20090 grams in 56 days of feeding in this experiment. The dynamics of the average weekly increase in the weight of piglets of the first treatment group is given in Table 2.

Table 2. Dynamics of the average weekly increase in pig weight in the first treatment group with the addition of Bio-Mos prebiotics in a concentration of 0.1%.

Terms	♀ $\bar{X} \pm S_{\bar{X}}$	*Vk*	♂ $\bar{X} \pm S_{\bar{X}}$	*Vk*	% $\Delta\bar{X}$	t_{exp}:
Initial weight	7319.0 ± 83.1	3.58	7222.5 ± 127.9	5.60	1.34	0.633 *ns*
7	7497.0 ± 158.5	6.69	7377.5 ± 212.9	9.13	1.62	0.452 *ns*
14	8401.0 ± 294.8	11.10	8041.5 ± 343.9	13.52	4.47	0.794 *ns*
21	10111.0 ± 415.7	13.00	9419.0 ± 555.8	18.66	7.35	0.997 *ns*
28	13175.0 ± 585.9	14.06	11765.0 ± 714.9	19.22	11.98	1.525 *ns*
35	15370.0 ± 684.4	14.08	13665.0 ± 999.2	23.12	12.48	1.408 *ns*
42	18480.0 ± 805.4	13.78	15975.0 ± 1022.0	20.23	15.68	1,925 *ns*
49	21540.0 ± 1024.9	15.05	18680.0 ± 1232.2	20.86	15.31	1,784 *ns*
56	23756.0 ± 1055.8	14.05	20770.0 ± 1370.7	20.86	14.38	1,726 *ns*

Insight into Table 2 shows that the difference in average weight between male and female piglets in terms of measurements differs from 1.34% (initial weight) to 15.68% (day 42). The significance of the difference determined by the t-test between male and female piglets of the first treatment group shows that it is not statistically significant at any time. However, in the sixth and seventh measurement periods (days 42 and 49), the average weight of female piglets is higher than that of male piglets by over 15%, which can be considered indicative according to the research goal. However, in the eighth measurement period (day 56) this difference is less than 15% and as it is not statistically significant for the final analysis of the effect of applied feeding models, we can conclude that piglets in the first treatment group, regardless of sex, for 56 days of this experiment achieved an average weight of 22263 grams. When we look at the increase, from the results of the analysis of other researchers, which included 24 experiments, performed by *Hooge (2003)*, it is noticeable that the use of prebiotics led to an improvement of 1.88% in the groups that used this additive in the diet in relation to groups without added additives.

The dynamics of the average weekly increase in the weight of piglets of the second treatment group is given in the Table 3.

Table 3. Dynamics of the average weekly increase in pig weight in the second treatment group with the addition of Bio-Mos prebiotics in a concentration of 0.2%.

Terms	♀		♂		% $\Delta\bar{X}$	t_{exp}:
	$\bar{X} \pm S_{\bar{x}}$	*Vk*	$\bar{X} \pm S_{\bar{x}}$	*Vk*		
Initial weight	7188.0 ± 103.2	4.54	7350.5 ± 121.8	5.24	2.26	1.018 *ns*
7	7181.0 ± 139.4	6.14	7280.5 ± 155.8	6.77	1.38	0.476 *ns*
14	7814.5 ± 179.6	7.26	7988.0 ± 249.2	9.86	2.22	0.565 *ns*
21	9209.0 ± 222.8	7.65	9573.0 ± 416.1	13.70	3.95	0.771 *ns*
28	10545.0 ± 310.6	9.31	10780.0 ± 606.9	17.80	2.23	0.345 *ns*
35	12445.0 ± 383.6	9.75	13290.0 ± 874.2	20.80	6.79	0.885 *ns*
42	14315.0 ± 454.4	10.04	14935.0 ± 1102.1	23.34	4.33	0.520 *ns*
49	16625.0 ± 541.9	10.31	17605.0 ± 1467.5	26.36	5.89	0.626 *ns*
56	18825.0 ± 583.3	9.80	19760.0 ± 1656.6	26.51	4.97	0.532 *ns*

Insight into Table 3 shows that the difference in average weight between male and female piglets in terms of measurements differs from 1.38% (day 7) to 6.79% (day 35). The significance of the difference determined by the t-test between male and female piglets of the second treatment group shows that it is not statistically significant at any time. For the final analysis of the effect of the applied feeding models, we can state that the piglets in the second treatment group,

regardless of sex, achieved an average weight of 19292.5 grams in 56 days of feeding in this experiment. *Newman (1999)* performed two experiments in which the aim was to examine the effect of mannan-oligosaccharides on the production results of broilers, where the preparation Bio-Mos was used. Reviewing the results, we notice that the use of Bio-Mos in the amount of 0.1 and 0.2%, respectively, results in a statistically significant increase in growth in the first experiment by 4.8% and in the second 5.5%.

The dynamics of the average weekly increase in the weight of piglets of the third treatment group is given in Table 4.

Table 4. Dynamics of the average weekly increase in the weight of piglets of the third treatment group with the addition of Bio-Mos prebiotics in the concentration of 0.3%.

Terms	♀		♂		% $\Delta\bar{X}$	t_{exp}:
	$\bar{X} \pm S_{\bar{X}}$	*Vk*	$\bar{X} \pm S_{\bar{X}}$	*Vk*		
Initial weight	7321.5 ± 90.25	3.89	7246.5 ± 144.6	6.31	1.03	0.440 *ns*
7	7456.5 ± 108.2	4.56	7441.0 ± 242.5	10.31	0.20	0.058 *ns*
14	8116.0 ± 226.3	8.82	8430.0 ± 337.1	12.64	3.72	0.773 *ns*
21	9864.5 ± 361.3	11.58	10302.5 ± 506.1	15.53	4.44	0.704 *ns*
28	12220.0 ± 456.2	11.81	12925.0 ± 765.9	18.74	5.77	0.791 *ns*
35	15075.0 ± 596.0	12.50	16040.0 ± 953.6	18.79	6.40	0.858 *ns*
42	15855.0 ± 801.1	15.98	18165.0 ± 1110.5	19.33	14.57	1.687 *ns*
49	19020.0 ± 734.1	12.21	20865.0 ± 1253.4	18.99	9.70	1.270 *ns*
56	21985.0 ± 881.7	12.68	24130.0 ± 1263.7	16.56	9.76	1.392 *ns*

Insight into Table 4 shows that the difference in average weight between male and female piglets in terms of measurements varies from 0.2% (day 7) to 14.57% (day 42). The significance of the difference determined by the t-test between male and female piglets of the third treatment group shows that it is not statistically significant at any time. For the final analysis of the effect of the applied feeding models, we can state that the piglets in the third treatment group, regardless of sex, achieved an average weight of 23057.5 grams in 56 days of feeding in this experiment. *Živković et al. (2011)* examined the influence of Bio-Mos in the diet of sows and piglets, where they observed a higher increase of 4.4% in experimental groups of animals that included this prebiotic in their diet. In contrast, data from an experiment conducted by *Biagi (2007)* show that there is insufficient evidence that prebiotics can significantly improve growth in weaned piglets.

The dynamics of the average weekly increase in the weight of piglets of the fourth treatment group is given in Table 5.

Table 5. Dynamics of the average weekly increase in the weight of piglets of the fourth treatment group with the addition of Bio-Mos prebiotics in a concentration of 0.4%.

Terms	♀ $\bar{X} \pm S_{\bar{x}}$	*Vk*	♂ $\bar{X} \pm S_{\bar{x}}$	*Vk*	% $\Delta\bar{X}$	t_{exp}:
Initial weight	7049.5 ± 137.4	6.16	7366.0 ± 123.2	5,29	4.49	1.715 *ns*
7	7239.0 ± 215.6	9.42	7500.5 ± 193.3	8,15	3.61	0.903 *ns*
14	7954.0 ± 261.6	10.40	8230.5 ± 278.4	10,69	3.48	0.785 *ns*
21	9501.5 ± 354.4	11.79	9792.2 ± 384.4	12,41	3.06	0.556 *ns*
28	12150.0 ± 466.6	12.14	12320.0 ± 444.4	11,41	1.40	0.264 *ns*
35	14400.0 ± 729.3	16.02	14885.0 ± 542.3	11,52	3.36	0.534 *ns*
42	17065.0 ± 711.9	13.19	17110.0 ± 601.1	11,11	0.26	0.048 *ns*
49	20765.0 ± 884.1	13.46	20745.0 ± 658.8	10,04	0.01	0.018 *ns*
56	22955.0 ± 914.3	12.59	23260.0 ± 800.1	10,88	1.33	0.251 *ns*

Insight into Table 5 shows that the difference in average weight between male and female piglets in terms of measurements differs from 0.01% (day 49) to 4.49% (initial weight). The significance of the difference determined by the t-test between male and female piglets of the fourth treatment group shows that it is not statistically significant at any time. For the final analysis of the effect of the applied feeding models, we can state that the piglets in the fourth treatment group, regardless of sex, achieved an average weight of 23107.5 grams in 56 days of feeding in this experiment.

The presence of *Escherichia coli* in the faeces, as a consequence of applied dietary treatments

It is believed that most diarrhea and indigestion in piglets are caused by different serotypes of the bacterium *Escherichia coli*. Infections caused by the bacterium *Escherichia coli* most often occur in young categories of pigs, in new-born piglets on suckling in the first days of life, weaned piglets, and immediately after weaning when the diet of concentrated nutrients begins. The transition from dairy to a concentrated diet and an overloaded digestive tract, after ingestion, result in excessive reproduction of *Escherichia coli* strains in the intestines. Prebiotics in this period have a significant role in reducing the number of *Escherichia coli* bacteria in the digestive tract, and preventing the occurrence of diarrhea and other indigestion to which this category of pigs is susceptible. *Escherichia coli* was analysed after switching to the grower diet. The results of the analysis are shown in Table 6.

Table 6. Number of *Escherichia coli* from faecal samples by treatment groups (× 10^8)

Treatment	Group	Day 35	Day 46	Day 55
Control	A	400	500	400
	B	250	300	300
I treatment group	A	7.5	1.5	1.5
	B	8.0	2.0	1.5
II treatment group	A	2.5	2.0	1.5
	B	2.5	1.0	1.0
III treatment group	A	2.0	2.0	1.5
	B	2.5	2.0	2.0
IV treatment group	A	2.5	2.0	2.0
	B	2.5	2.5	2.0

Table 7. Average for A and B (×10^8)

Treatment	Day 35	Day 46	Day 55
Control	325	400	350
I treatment group	7.75	1.75	1.50
II treatment group	2.50	1.50	1.25
III treatment group	2.25	2.00	1.50
IV treatment group	2.50	2.25	2.00

A review of Tables 6 and 7 shows that the content of *Escherichia coli* in the control group is higher than in the treatment groups fed with Bio-Mos prebiotic by at least 42 to 260 times, and at most by 52 to 320 times. Given such a drastic reduction in the content of *Escherichia coli* in the faeces of treatment groups fed with Bio-Mos prebiotic, such a phenomenon does not have to be proven by any statistical testing. Results similar to the results in this paper have been obtained and presented by many authors. *Deng et al. (2007)* found in their research that the inclusion of prebiotics in the diet of piglets leads to an increase in the number of *Lactobacillus* and a decrease in the number of *Escherichia coli* in the ileum and colon of piglets. The results in accordance with the above were also presented by *Liu et al. (2008)* in which they state that the addition of prebiotics to the diet of weaned piglets successfully increases the number of *Lactobacillus* bacteria and reduces the number of *Escherichia coli* in faecal samples taken twice.

In the treatment groups fed with Bio-Mos prebiotic, only the treatment group I was singled out in the first term of analysis (7.75), which can be related to the fact that the smallest amount of prebiotics (0.1%) was used here, as well as that in further terms of analysis the content of *Escherichia coli* in the faeces, between this treatment group and other treatment groups with increased content of Bio-Mos prebiotics, there are practically no differences. Thus, the lowest concentration of prebiotics of 0.1% had a positive effect on the reduction of the number of *Escherichia coli* bacteria. In support of this statement, a noticeable decrease in the

content of *Escherichia coli* in the faeces with the length of the diet with Bio-Mos prebiotic can be pointed out: 3.75 → 1.87 → 1.56, i.e. expressed by the base index: 100% → 49.87% → 41.6%. The performed analysis clearly confirms the influence of Bio-Mos prebiotics on the content of *Escherichia coli* in the faeces of piglets in this experiment.

Conclusion

The analysis of the significance of the differences in the average weight between female and male piglets observed at the level of weekly dynamics shows that the differences that occurred can be assessed as random, not argued by the statistical significance. Although without statistical significance, observing the results of all treatment groups, it can be noticed that the best results in terms of the achieved average weight are given by the highest used concentrations of prebiotics in food, 0.4% but also 0.3%. From the analysis of faeces for the number of *Escherichia coli* bacteria, it is clear that the number of bacteria differs significantly between the treatment groups and the control group. The preparation had a positive effect in terms of reducing the number of *Escherichia coli* bacteria in the digestive tract of piglets in all treatment groups, drastically, by 42 to 320 times. A decrease in the number of bacteria with long-term use was also observed, and according to the sampling stages, this share decreased from 100% over 49.87% to 41.6% in the last sampling period. The share, that is, the percentage of prebiotics in food is not of special importance, because even the lowest concentration used gave a positive result in reducing the number of *Escherichia coli*. It is recommended that the use of this preparation should probably be longer and in higher concentrations up to 0.3%, and that in the next phase of pig breeding, it is likely that there will be a significant manifestation of the positive effects of the additive used on production parameters.

Uticaj prebiotika u ishrani prasadi na telesnu masu i sadržaj *Escherichia coli* u fecesu

Mirjana Delić-Jović

Rezime

Cilj istraživanja je bio da se utvrdi uticaj prebiotika u ishrani prasadi po zalučenju na porast prosečne mase eksperimentalnih životinja, između ženske (♀) i muške (♂) prasadi, dinamički po nedeljama eksperimenta, kao i na prisustvo bakterije *Escherichia coli* u fecesu. Eksperimentom su bila obuhvaćena prasad po zalučenju, starosti oko 26 dana. Korišćena je F1 generacija, koja je nastala ukrštanjem krmača

landrasa i nerastova pijetrena. Ishrana prasadi trajala je 56 dana (8 nedelja). Formirane su četiri grupe, svaka grupa bila je sastavljena od 10 prasadi (5 ženskih i 5 muških) sa različitim koncentracijama prebiotika i kontrolna, u dva ponavljanja, ukupno 100 eksperimentalih životinja. U ishrani su korišćene krmne smeše koje su se sastojale od istih hraniva. Kontrolne grupe (O-A i O-B) su hranjene krmnom smešom bez dodavanja aditiva, grupe (I-A i I-B) sa dodatkom prebiotika Bio-Mos-a, u koncentraciji od 0,1%, grupe (II-A i II-B) sa 0,2%, grupe (III-A i III-B) sa 0,3% i grupe (IV-A i IV-B) su bile hranjene sa dodatkom prebiotika u koncentraciji od 0,4%. Zaključeno je da razlike koje su utvrđene u prosečnoj masi između ♀ i ♂ prasadi ni u jednom terminu merenja nisu bile statistički značajne. Ipak, najbolje rezultate u pogledu ostvarene prosečne mase dale su najviše korišćene koncentracije prebiotika u hrani, 0,4 % i 0,3 %. Preparat je ispoljio pozitivan efekat u pogledu smanjenja broja bakterije *Escherichia coli* u digestivnom traktu prasadi kod svih tretmanskih grupa i to za 42 do čak 320 puta.

Ključne reči: prase, prebiotik, masa, *Escherichia coli*

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THE EFFECT OF FISH MEAL IN THE NUTRITION OF WEANED PIGLETS

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Abstract: The effects of the use of fish meal in the nutrition of weaning piglets were observed. The trial included 80 weaned piglets of the same genotype (Large White x Danish Landrace) distributed in two feeding treatments. In the first period of the experiment, animals were fed during 10 feeding days, with a pre-starter mixture containing 21.1/20.8% of the crude protein. The second period lasted for 25 days and piglets were fed with mixture containing also 21.0/21.1% of the crude protein. And in the final period of the experiment, which lasted 22 feeding days, the meals were formulated to contain 19.5/19.9% of the crude protein. The control group was fed with standard farm mixtures, while the trial group were fed with mixtures containing 4/3/2% of fish meal. The obtained results showed that the use of fish meal resulted in better average daily gain and feed conversion in starter period (27-51 day).

Key words: diet, growth promoters, post-weaning period

Introduction

Fish meal is a biproduct of animal origin and is a source of high-quality protein for animal feed. Currently, fish meal is manufactured from species that are caught in commercial fishing and not adequate for human consumption. Inclusion of high-quality protein sources (fish meal) is important for pig diets to manage digestive disturbances in the weaning period.

According to the decision of the EU Commission 9/2001, all the mixtures which contain fish meal can only be produced in feed mills which don't produce or process any other feeds for ruminants or if they are specialized for this purpose. And also they need proper licence from authorised institutions (*Sardi et al., 2005*). This decision has led to some negative marketing in regard of using animal proteins in livestock feed, which led to further research of the possibility to excluding fish meal from mixtures for pigs and implementing some substitutes.

The weaning period is always stressor to young pigs that typically results in lower feed intake and a decrease in bodyweight for several days immediately after weaning (*Hötzel et al., 2011*). With a loss of bodyweight, an ileal environment is favorable for the colonization of bacteria resulting in post-weaning diarrhea syndrome (*Tsiloyiannis et al., 2001*). It is important in the immediate post-weaning period to include high-quality ingredients such as fish meal in diets (*DeRouchey et al., 2010*), which are proven to increase feed intake and growth performance (*Berrocoso et al., 2012*).

There is increased concern about overfishing of wild capture fisheries, and as of 2011, 28.8% of the world's fish species were overfished (*FAO, 2012*). This over-harvest has included species used for fish meal production and when coupled with increasing global demand for fish meal has led to an unsustainable situation (*Olsen and Hasan, 2012*). Soybean meal is the mostly used protein source in pig diets, but it has it's limitations in young pig diets due to anti-nutritional factors, which influences post-weaning diarrhea syndrome (*Friesen et al., 1993*). Also, it is important to find alternative high-quality protein sources that are more economically efficient and contain minimal anti nutritional factors.

A common practice that inclusion of high quality protein ingredients in piglet diets is the result of their immature and defective digestive system, which can minimize the side effects on digestive function and growth rate in the period of weaning *(Che et al., 2012; Sinn et al, 2016).*

Fish meal has similar nutritive characteristics like some other alternatives to fish meal, like the plant feed - Ekofish meal used in the nutrition of weaned piglets (*Adamović et al., 2006*), sows and piglets (*Živković et al., 2007a*).

Objective of this paper was to investigate the effects of use ultrapure, high protein feed fish meal in diets for weaned piglets.

Material and Methods

The trial included 80 piglets of the same genotype (Large White x Danish Landrace) distributed in two feeding treatments (Table 1). Immediately after the piglets were weaned, groups of 10 piglets were formed on the basis of uniform initial weight, taking into account that in each group the sex ratio is the same. There were 4 repetitions per treatment. All piglets were placed in solid wall boxes, with lattice floor, each containing 10 feeding places. Average initial weight of piglets was 7.64 kg. All piglets came from 8 different mothers, and same father. There were three feeding mixtures for the whole trial period (Table 1.) In the first period of the experiment, animals were fed during 10 feeding days, with a pre-starter mixture containing 21.1/20.8% of the crude protein. The second period lasted for 25 days and piglets were fed with mixture containing also 21.0/21.1% of the crude protein. And in the final period of the experiment, which lasted 22

feeding days, the meals were formulated to contain 19.5/19.9% of the crude protein.

The first group of piglets, control, was fed with standard farm mixture and the other group of piglets with mixtures containing fish meal (Table 2). Food and water were *ad libitum*.

Table 1 . Composition of diets for weaned piglets in the trial

	Pre-starter, Day 18-27		Starter, Day 27-51		Grower, Day 52-73	
Group	C(control)	T(trial)	C(control)	T(trial)	C(control)	T(trial)
Ingredients, g/kg						
Maize	265.1	282.0	270.3	283.0	312.9	313.5
Barley	100.0	100.0	100.0	100.0	187.5	187.5
Triticale	100.0	100.0	100.0	100.0	65.0	65.0
Soybean meal	334.0	278.0	379.0	337.0	100.0	100.0
Soybean semolina	-	-	-	-	284.0	264.0
Milk replacer	50.0	50.0	-	-	-	-
Whey	40.0	40.0	40.0	40.0	-	-
Sunflower oil	10.0	10.0	10.0	10.0	-	-
Fish meal	-	40.0	-	30.0	-	20.0
Mineral–vitamin premix 1*	100.0	100.0	100.0	100.0	-	-
Mineral–vitamin premix 2**	-	-	-	-	50.0	50.0
L-Lysine	0.5	-	0.4	-	0.5	-
DL-Methionine	0.4	-	0.3	-	0.1	-
Calculated nutrient composition, g/kg of feed						
Crude protein	211.11	208.60	210.10	211.10	195.10	199.00
Lysine	12.50	12.50	12.30	12.30	11.10	11.20
Methionine	3.80	3.70	3.50	3.50	3.10	3.30
Cysteine	3.50	3.30	3.10	3.50	3.40	3.40
Threonine	8.00	8.20	7.90	8.20	7.60	7.80
Tryptophan	2.60	2.60	2.70	2.60	2.40	2.40
Crude fibre	34.20	31.00	37.10	34.80	39.70	38.60
Crude fat	29.30	32.80	29.50	32.10	69.00	67.20
Calcium	12.10	12.10	12.11	12.11	10.82	10.82
Phosphorus	7.36	7.36	7.37	7.37	7.55	7.55
DE content, MJ/kg	15.03	15.07	15.01	15.04	16.68	16.64

*The commercial premixes (10% premix for piglets).
*The commercial premixes (5% premix for piglets).

Table 2. Nutritive value of the Fish meal used in the experiment

Composition	Fish meal
ME,MJ/kg	13.20
Moisture, %	8.0
Crude protein, %	62.0
Crude fiber, %	3.0
Ash, %	4.0
Calcium, %	0.90
Phosphorus total, %	0.54
Sodium, %	0.16
Some essential amino acids, g/16 gN :	
Lysine	7.82
Methionine + cystine	4.00
Tryptophane	1.06
Threonine	3.96

During the starter and grower period, the following production indicators were monitored: body weight, average daily gain, average daily food consumption and feed conversion. The data obtained were processed using the software package "STATISTICA" (*Stat Soft Inc, 2012*). ANOVA was used while the Tukey test served to determine the statistical significance of the differences between individual means values.

Results and Discussion

Production performances (Table 3.) were shown that during the whole trial period, it was found that there were no significant differences in average feed intake, daily gain and feed conversion. Statistical significance was noted in ADG and FCR during starter period, trial group had better average daily gain (15.64%) and feed conversion (13.63%). In the grower period only statistical difference were noted for ADG where control, group had better ADG for 9.37%. Durring whole trial period there were no mortalities.

Table 3. Production performance

	Treatments		SEM	p
	C	T		
Starter period (27-51d)				
FI, g/d	501.76	510.61	0.098	0.746
ADG, g/d	286.72[b]	331.57[a]	0.145	p<0.05
FCR, g/g	1.75[a]	1.54[b]	0.123	p<0.05
Grower period (52-73d)				
FI, g/d	963.89	977.98	0.089	0.566
ADG, g/d	465.65[a]	436.60[b]	0.163	p<0.05
FCR, g/g	2.07	2.24	0.137	0.112
Whole period (27-73d)				
FI, g/d	714.29	720.14	0.046	0.899
ADG, g/d	370.10	377.04	0.076	0.788
FCR, g/g	1.93	1.91	0.029	0.856

SEM, Standard error of the means; FI, feed intake; ADG, average daily gain; FCR, feed conversion rate; [a, b,] In a row, the least squares means with a different superscript differ significantly (p<0.05)

Some researchers have concluded that piglets fed with products based on soybean proteins can progress almost equally to piglets fed with the fish meal (*Min et al., 2003; Sardi et al., 2005*). Soybean meal are a very good substitute to fish meal (*Ebert et al., 2005a*) and because of arginine could be even better than whey protein (*Ebert et al., 2005b*), but they are pretty inferior compared to casein (*Junghans et al., 2004*).

Adding lecithin and enzyme to diets based on soybean meal could not reach the same level of feed utilization as the one based on fish meal (*Kovčin et al., 2005*). According to *Sardi et al. (2005)* fish meal used in weaned piglet diets can be replaced by almost same amounts of vegetable protein. Some newer studies (*Chia et al., 2019*) even include insect meal as alternative to fish meal. *Živković et al. (2007b)*, have shown that use of plant based fish meals could be as efficiant as fish meal, and beneficial for suckling and weaned piglets.

Jeong and Kim (2015) found no differences on growth performance, in weaned pigs when other products replaced half of the fish meal in diets. Fish meal from different sources can have different effect growth performance (*Cho et al., 2012*). *Jones et al.(2015)* concluded that adding 3% fish meal to diet improved ADG, and adding 6% improved both ADG and FCR.

Fish meal is widely used in weaned piglet diets as a highly digestible protein with excellent amino-acid profile and very high level of vitamins and minerals (*Sun et al. 2009*).

Conclusion

Results obtained in starter period are promising, and it could be recommended that fish meal can be used in the first 25 days after weaning. However, results obtained in the second part of the experiment could not justify usage of fish meal over a less expensive soybean meal. In the last ten years many researchers and companies searched for adequate substitute for fish meal. However recent studies suggest using fish meal and it superiority in some aspects, over substitutes. So maybe the future holds reinstating fish meal as main animal feed for young pigs. Also further investigation, considering influence of fish meal on post-weaning diarrhea syndrome, should be done.

Efekat korišćenja ribljeg brašna u ishrani zalučene prasadi

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Rezime

Ispitivani su uticaji korišćenja ribljeg brašna u ishrani prasadi u odgoju. Ogled je sproveden na 80 prasadi, genotipa Veliki jorkšir x Danski landras, podeljenih u dve grupe tokom celog perioda istraživanja. U prvom periodu eksperimenta, životinje su hranjene tokom 10 dana, predstarter smešom koja je sadržala 21,1/20,8% sirovog proteina. Drugi period je trajao 25 dana, a prasad su hranjeni smešom koja je sadržala 21,0/21,1% sirovog proteina. U poslednjem periodu eksperimenta, koji je trajao 22 dana, obroci su formulisani tako da sadrže 19,5/19,9% sirovih proteina. Kontrolna grupa je hranjena sa standarnim farmskim smešama, dok je ogledna grupa hranjena smešama koje su sadržale riblje brašno u koncetraciji od 4/3/2%. Dobijeni rezultati su pokazali da korišćenjem ribljeg brašna dolazi do poboljšanja prirasta i konverzije hrane u periodu nakon zalučenja (27-51 dan).

Ključne reči: ishrana, promoter porasta, period nakon zalučenja

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CHARACTERISTICS AND VARIABILITY OF UDDER SCORES OF SIMMENTAL FIRST CALVING HEIFERS

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Abstract: Visual assessment and recognition of milk performance traits of cows are preliminary indicators of milk yield, longevity, as well as reproductive abilities of the animal, which is very important from the aspect of economy of milk production. Deficiencies in udder traits lead to poorer production, difficult milking and premature weaning of cows from the herd. The paper examines the frequency of desirable scores for a certain trait in first-calving heifers distinguished by way of keeping (heads reared by individual producers/holdings and heads reared on the farm) and by origin (domestic and imported animals), as well as the influence of these two factors on the observed traits. Five udder traits were analyzed: front udder length, rear udder length, rear udder height, central ligament, and udder depth on a total of 954 first-calving Simmental heifers. Observed by the way of rearing, higher frequency of desirable scores for all udder traits were achieved by cows reared on the farm, while according to the origin of cows, higher frequency of desirable scores for all udder traits was achieved by imported cows compared to domestic cows. The influence of factors of housing/keeping and origin, examined by $\chi 2$ test on all examined linear scores (frequency of scores) of udder traits was statistically very highly significant ($p \leq 0.001$), while the analysis of variance (F test) showed very high significance ($p \leq 0.001$) of interaction of origin and method of rearing on the traits of the central ligament and the udder depth, and significance ($p \leq 0.05$) on the height of the rear udder, however, no statistical significance ($p > 0.05$) of this interaction was established on other linear scores of udder traits (length of the front and rear udder).

Key words: Simmental, udder, rear udder height, front udder length, central ligament

Introduction

The evaluation of the exterior of animals is performed on the basis of knowledge of the structure and function of individual organs as well as the most important relationships between individual body parts.

The assessment of the body development of cattle is a critical assessment of whether the individual animal in its overall appearance as well as individual parts of the body, guarantees, in addition to good health, high production performance, i.e. its longevity. The inclusion of a linear type scores contributes to the reliability of the evaluation of the breeding value of cows, which has a positive effect on the overall effects of selection and production success (*Pantelić et al., 2006*).

Linear evaluation includes the evaluation of each predicted trait in its biological extremes, according to a scale ranging from 1 to 9 in the evaluation chart, and it should be emphasized that the highest score (9) is not the most favourable for each trait, because for some traits average grades (5) are the best *(Petrović and Pantelić, 2015).*

Živanović (2002) has examined the variability of linearly assessed traits of type and milk yield of first-calving heifers of Black and White breed on a sample of 2,976 cows of PK "Belgrade". Linear assessment of the type was performed in the period from 30 to 150 days from the beginning of lactation. The score system included 14 traits, i.e. 6 traits of body development and 8 udder traits. The average values of the obtained scores ranged from 5.28 to 7.15 for the body development, and 5.06 to 7.02 for the udder traits.

The cow's udder is one of the most important criteria that can be used to predict production performance *(Vukašinović et al., 1995).* The trait of udder height is positively related to better genetic potential in the first lactation. The conformation of the udder and the teat position are related to the health of the udder and the efficiency of the automatic milking (with the use of milking machines). Also, cows with a high scores for body development traits of live animals in the herd were not associated with health problems.

Rogers et al. (1981) find that the depth of the udder and the rear teat are the traits most closely associated with the longevity of cows. Selection based on body development traits and milk performance could result in higher genetic gains in milk yield than selection based on individual milk performance.

The importance of the structure and udder attachment and other traits on milk yield and longevity of cows has been examined by *Sawa et al. (2013),* stating that life expectancy is in the strongest correlation with udder traits (r = 0.22), followed by results for type and conformation of both legs and feet (r = 0.13) and individual traits such as udder width and dairy character (r = 0.14). The traits of the udder, legs and feet together exhibit the highest positive effect on longevity (r =

0.11), and among the individual traits, the position of the udder (r = 0.14) and the front udder attachment (r = 0.10).

Examining the influence of individual traits and their scores on cow productivity in Ethiopia, *Yaman et al. (2015)* establish a correlation between individual traits and milk yield of cows and expressed this through the Pearson coefficient, as follows: for udder length (r = 0.63), teat length (r = 0.53) and body length (r = 0.65). Of the qualitative traits, about 53.34% of the owners interviewed in this dairy farm survey stated that udder size and teat placement are considered to be the main traits for selection of dairy cows.

According to the research conducted by *Gulinski et al. (2005),* the conformation traits most closely related to milk yield include udder width (r = 0.26), angularity (r = 0.21), overall type and conformation score (r = 0.19). *Chabuz et al. (2003)* show that milk yield, on a scale of 100 points, is most closely related to the conformation score (r = 0.43), then hip height (r = 0.31) and udder traits, especially udder width (r = 0.49) and front udder length (r = 0.35).

Material and Methods

Basic data on the traits of body fundament, as well as data on the origin of all examined cows, were collected in cooperation with the dairy farm "Lazar" Blace, which housed part of the animals covered by this study. For animals reared on farms of individual producers (holdings), data on these traits were collected in cooperation with the breeding organizations, which implement the breeding program in the Toplica district.

The animals (n = 954) included in the analysis of morphometric traits were divided into two groups based on the method of rearing, and two groups based on the origin, as follows:

Based on the method of rearing:

Group 1: animals reared by individual producers (n = 504);

Group 2: animals reared on the farm (n = 450);

Based on the origin:

Group 1 animals of domestic origin (n = 718);

Group 2: imported animals (n = 236).

All udder traits were linearly assessed after the first calving, namely: front and rear udder length, rear udder height, central ligament and udder depth.

The processing of the collected data consisted of determining the frequency for each score individually, the frequency of the preferred scores for all udder traits, and comparing the obtained frequencies by groups. Subsequently, the influence of method of rearing (applied $\chi 2$ test) and the influence of the origin of animal (applied $\chi 2$ test) on the frequency of linear scores for each udder trait were

examined, while the analysis of variance examined the influence of rearing and origin, as well as their interaction on all linear scores, with the following model:
Model with fixed influence of rearing and origin and their interaction:

$Y_{ij}=\mu+N_i+P_j+NP_{ij}+e_{ijk}$

- Y_{ij}: tested trait,
- μ: population average for a given trait,
- N_i: fixed influence of the i way of rearing (i = 1,2),
- P_j: fixed influence of j origin (j = 1,2),
- NP_{ij}: the influence of factor interaction (rearing and t origin),
  eij: random error

For statistical data processing and application of the mentioned model, the software SPSS Statistics for windows, Version 23.0 was used.

Results and Discussion

Based on the results shown in Table 1, it can be concluded that the highest number of animals did not have the desired score (7,8,9) for the trait of the front udder length, however, imported animals (45.34%) and animals reared on the farm (45.11%) had a higher frequency of desirable scores than animals reared by individual producers (34.52%), and of domestic origin (37.60%). The origin of the animal and the method of rearing showed statistically very highly significant ($p\leq0.001$) impact on the frequency of scores for the front udder length, examined by the $\chi2$ test. Analysis of variance (F test) showed that the interaction of origin and method of rearing, and method of rearing individually did not have a significant impact ($p\leq0.05$) on the examined trait.

The frequency of desirable scores for the rear udder length was higher in farm reared cattle than in first – calving heifers reared by individual producers and amounted to 40.67%. Animals of imported origin also had a higher percentage of desirable scores (37.71%) than heads of domestic origin (33.70%).

Based on the $\chi2$ independence test, it was determined that there was a statistically very significant correlation ($p\leq0.001$) between the origin and scores for the rear udder length in first calving heifers, as well as the method of rearing and scores for the same trait. Analysis of variance (F test) showed that the method of rearing and the origin of the animals, as well as their interaction, did not have a statistically significant effect ($p> 0.05$) on the rear udder length.

Table 1. Linear scores and their frequencies for the traits front and rear udder length in Simmental first-calving heifers

Scores	Rearing						Origin					
	Animals reared by individual producers			Animals reared on the farm			Domestic animals			Imported animals		
	N	%	% group	N	%	% group	N	%	% group	N	%	% group
FRONT UDDER LENGTH												
1	0	0.00		0	0.00		0	0.00		0	0.00	
2	0	0.00	0.20	0	0.00	1.56	0	0.00	0.28	0	0.00	2.54
3	1	0.20		7	1.56		2	0.28		6	2.54	
4	3	0.60		26	5.78		4	0.56		25	10.59	
5	144	28.57	65.28	142	31.56	53.33	245	34.12	62.12	41	17.37	52.12
6	182	36.11		72	16.00		197	27.44		57	24.15	
7	**166**	**32.94**		**159**	**35.33**		**268**	**37.33**		**57**	**24.15**	
8	**8**	**1.59**	**34.52**	**44**	**9.78**	**45.11**	**2**	**0.28**	**37.60**	**50**	**21.19**	**45.34**
9	**0**	**0.00**		**0**	**0.00**		**0**	**0.00**		**0**	**0.00**	
χ2 test												
Rearing χ2=92.707*** p=0.000							Origin χ2=238.545*** p=0.000					
F test												
Rearing F=0.030 nz p=0.861							Origin F=4.247* p=0.040					
Rearing x Origin F=1.047nz											p=0.307	
REAR UDDER LENGTH												
1	0	0.00		0	0.00		0	0.00		0	0.00	
2	0	0.00	0.19	0	0.00	0.66	0	0.00	0.28	0	0.00	0.85
3	1	0.19		3	0.66		2	0.28		2	0.85	
4	3	0.60		25	5.56		4	0.56		24	10.17	
5	155	30.75	70.44	165	36.67	58.67	260	36.21	66.02	60	25.42	61.44
6	197	39.09		74	16.44		210	29.25		61	25.85	
7	**141**	**27.98**		**148**	**32.89**		**241**	**33.57**		**48**	**20.34**	
8	**7**	**1.39**	**29.37**	**35**	**7.78**	**40.67**	**1**	**0.14**	**33.70**	**41**	**17.37**	**37.71**
9	**0**	**0.00**		**0**	**0.00**		**0**	**0.00**		**0**	**0.00**	
χ2 Test												
Rearing χ2=90.494*** p=0.000							Origin χ2=194.253*** p=0.000					
F Test												
RearingF=0.018nz p=0.894							Origin F=1.949 nz p=0.163					
Rearing x Origin F=0.084ns											p=0.771	

***- p≤0,001; ** - p≤0,01; * - p≤0,05; ns - p>0,05

In the analysis the rear udder height, central ligament and udder depth, by groups (Table 2), the lowest percentage of cows with favourable scores for the rear udder height were domestic cows and cows reared by individual producers (about 29%), then cows reared on the farm (34.00%) and the highest percentage with favourable scores for the rear udder height were cows originating from import (38.56%).

By testing the frequencies with the $\chi 2$ test, it was established that there was statistically very highly significant variation ($p \leq 0.001$) of the scores for the rear udder height under the influence of the rearing of cows and their origin. Analysis of variance (F test) revealed a highly significant influence of the method of rearing and the origin of the animals ($p \leq 0.01$), while the interaction of the origin and the method of rearing had statistically significant ($p < 0.05$) effect on the rear udder height.

The frequency of desirable scores for the central ligament trait was significantly higher in farm-reared cattle (30.22%) than in cattle reared on individual households where only 23.41% of first-calving heifers were given any of the desirable scores. If we look at the observed population on the basis of the origin, it can be stated that the frequency of desirable scores was significantly higher for animals originating from import (40.25%) than for animals originating from domestic breeding, where 22.14% of first-calving heifers were given desirable scores for the central ligament trait.

The influence of the origin and method of rearing of animals was statistically very highly significant ($p \leq 0.001$) on the frequency of scores for the central ligament, examined by the $\chi 2$ test. Analysis of variance (F test) also revealed very highly significant influence ($p \leq 0.001$) of the method of rearing, the origin of the animal and their interaction.

By examining the trait of udder depth, it can be stated that the highest number of animals had desirable scores (7,8,9) only in case of animals originating from import (56.36%), while other groups had a lower percentage: farm-reared cattle (44.89%), cattle reared by individual producers (43.25%), and domestic cattle (39.97%). The influence of the origin of the animal and the way of rearing was statistically very highly significant ($p \leq 0.001$) on the frequency of udder depth scores, examined by the $\chi 2$ test. Analysis of variance (F test) also revealed very highly significant influence ($p \leq 0.001$) of the method of rearing, the origin of the animal and their interaction.

Table 2. Linear scores and their frequencies for the traits rear udder height, central ligament and udder depth in Simmental first-calving heifers

Scores	Rearing						Origin					
	Animals reared by individual producers			Animals reared on the farm			Domestic animals			Imported animals		
	% group	N	%	% group	% group	N	%	% group	% group	N	%	% group
REAR UDDER HEIGHT												
1	0	0.00		0	0.00		0	0.00		0	0.00	
2	0	0.00	1.19	0	0.00	2.22	0	0.00	1.67	0	0.00	1.69
3	6	1.19		10	2.22		12	1.67		4	1.69	
4	3	0.60		27	6.00		7	0.97		23	9.75	
5	145	28.77	69.25	156	34.67	63.78	231	32.17	68.94	70	29.66	59.75
6	201	39.88		104	23.11		257	35.79		48	20.34	
7	**130**	**25.79**		**122**	**27.11**		**209**	**29.11**		**43**	**18.22**	
8	**19**	**3.77**	**29.56**	**31**	**6.89**	**34.00**	**2**	**0.28**	**29.39**	**48**	**20.34**	**38.56**
9	**0**	**0.00**		**0**	**0.00**		**0**	**0.00**		**0**	**0.00**	
χ2 Test												
Rearing χ2=51.694*** p=0.000							Origin χ2=201.427*** p=0.000					
F Test												
RearingF=9.904** p=0.002							Origin F=8.450** p=0.004					
Rearing x Origin F=4.030*											p=0.045	
CENTRAL LIGAMENT												
1	0	0.00		0	0.00		0	0.00		0	0.00	
2	0	0.00	0.40	0	0.00	1.34	0	0.00	0.42	0	0.00	2.12
3	2	0.40		6	1.34		3	0.42		5	2.12	
4	1	0.20		32	7.11		1	0.14		32	13.56	
5	176	34.92	76.19	148	32.89	68.44	279	38.86	77.44	45	19.07	57.63
6	207	41.07		128	28.44		276	38.44		59	25.00	
7	**99**	**19.64**		**108**	**24.00**		**159**	**22.14**		**48**	**20.34**	
8	**15**	**2.98**	**23.41**	**26**	**5.78**	**30.22**	**0**	**0.00**	**22.14**	**41**	**17.37**	**40.25**
9	**4**	**0.79**		**2**	**0.44**		**0**	**0.00**		**6**	**2.54**	
χ2 Test												
Rearing χ2=53.294*** p=0.000							Origin χ2=271.482*** p=0.000					
F Test												
Rearing F=21.752*** p=0.000							Origin F=31.762*** p=0.000					
Rearing x Origin F=27.964***											p=0.000	

UDDER DEPTH												
1	0	0.00		0	0.00		0	0.00		0	0.00	
2	0	0.00	0.20	0	0.00	0.22	0	0.00	0.28	0	0.00	0.00
3	1	0.20		1	0.22		2	0.28		0	0.00	
4	1	0.20		9	2.00		3	0.42		7	2.97	
5	78	15.48	56.55	91	20.22	54.89	114	15.88	59.75	55	23.31	43.64
6	206	40.87		147	32.67		312	43.45		41	17.37	
7	**187**	**37.10**		**156**	**34.67**		**287**	**39.97**		**56**	**23.73**	
8	**20**	**3.97**	**43.25**	**39**	**8.67**	**44.89**	**0**	**0.00**	**39.97**	**59**	**25.00**	**56.36**
9	**11**	**2.18**		**7**	**1.56**		**0**	**0.00**		**18**	**7.63**	
χ2 Test												
Rearing χ2=24.091*** p=0.001							Origin χ2=297.142*** p=0.000					
F Test												
Rearing F=20.160*** p=0.000							Origin F=62.379*** p=0.000					
Rearing x Origin F=19.305***											p=0.000	

***- p≤0.001; ** - p≤0.01; * - p≤0.05; ns - p>0.05

Conclusion

By examining the scores for udder traits in Simmental first-calving heifers observed according to the method of rearing, the higher frequency of desirable scores for all udder traits was achieved by cows reared on the farm, while according to the origin of cows, the higher frequency of desirable scores for all body fundament traits were realized by imported animals in relation to cows of domestic origin. The influence of factors of rearing and origin examined by χ2 test on all examined linear scores (frequency of scores) of udder traits was statistically very highly significant (p≤0.001), while analysis of variance (F test) determined high significance (p≤0.001) of the interaction of origin and method of rearing on the udder depth and the central ligament, as well as significance (p≤0.05) of this interaction on the height of the rear udder, while the linear scores for the front and rear udder length did not show statistical significance (p> 0.05).

Karakteristike i varijabilnost ocena vimena prvotelki simentalske rase

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Rezime

Vizuelna procena i prepoznavanje mlečnih karakteristika krava su preliminarni pokazatelji mlečnosti, dugovečnosti, kao i reproduktivnih sposobnosti grla, što je veoma važno sa aspekta ekonomičnosti proizvodnje mleka. Nedostaci u osobinama vimena dovode do slabije proizvodnje, otežane muže i preranog isključenja krava iz zapata. U radu je ispitivana učestalost poželjnih ocena određene osobine kod prvotelki podeljenih po načinu držanja (grla kod individualnih proizvođača i grla sa farme) i podeljenih po poreklu (grla domaćeg odgoja i grla iz uvoza), kao i uticaj ova dva faktora na posmatrane osobine. Analizirano je pet osobina vimena: dužina prednjeg vimena, dužina zadnjeg vimena, visina zadnjeg vimena, centralni ligament i dubina vimena na ukupno 954 prvotelke simentalske rase. Posmatrano prema načinu držanja, veću frekvenciju poželjnih ocena za sve osobine vimena iskazane u ocenama ostvarile su krave sa farme, dok su prema poreklu krava, veću frekvenciju poželjnih ocena za sve osobine vimena iskazane u ocenama ostvarile krave poreklom iz uvoza u odnosu na krave domaćeg porekla. Uticaj faktora načina držanja i porekla grla ispitivani $\chi 2$ testom na sve isptitivane linearne ocene (frekvenciju ocena) osobina vimena bio je statistički vrlo visoko značajan ($p \leq 0,001$), dok je analizom varijanse (F test) utvrđena vrlo visoka značajnost ($p \leq 0,001$) interakcije porekla i načina držanja na osobine centralni ligament i dubina vimena, kao i značajnost ($p \leq 0,05$) na visinu zadnjeg vimena, dok na ostale linearne ocene osobina vimena (dužina prednjeg i zadnjeg vimena) nije ispoljila statističku značajnost ($p > 0,05$).

Ključne reči: simentalska rasa, vime, visina zadnjeg vimena, dužina prednjeg vimena, centralni ligament

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CHANGES OF SENSORY ATRIBUTES OF CHILLED VACUUM-PACKAGED COLD-SMOKED COMMON CARP (*CYPRINUS CARPIO*) AND COLD-SMOKED BIGHEAD CARP (*HYPOPHTHALMICHTHYS NOBILIS*) FILLETS

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Abstract: The aim of this research was to monitor changes of selected sensory properties and instrumental colour parameters (L*, a*, b*) of vacuum-packaged cold-smoked common carp (*Cyprinus carpio*) and cold-smoked bighead carp (*Hypophthalmichthys nobilis*) fillets during storage at 3 ± 0.5 °C and to determine the shelf life of the products. Sensory tests and instrumental color determination were performed on days 1, 7, 10, 12, 14, 15, and 16 of storage. All estimated sensory characteristics of common carp samples received significantly lower ($P < 0.05$) score on day 15. "Musty" odour of common carp samples detected on day 16 caused that odour score was below the acceptability limit of 2. On the last day of experiment, reduced intensity of pink cream colouring of carp muscle was observed together with softened texture and surface slime. A decrease of scores of the sensory attributes of cold-smoked bighead carp fillets was observed throughout the storage period. However, all estimated sensory characteristics were within the acceptability level. Significant increase ($p < 0.05$) of L* value was noted in both group of samples, while redness (a*) and yellowness (b*) remaid quit stable during the storage. Based on the sensory results, it was concluded that vacuum-packaged cold-smoked common carp samples remained acceptable for up to 15 days of storage, whereas vacuum packaged cold-smoked bighead carp samples remained unchanged until the end of the experiment.

Key words: cold-smoked fish, overall acceptability, shelf life, instrumental colour parameters

Introduction

Most of the wild fish and fish from aquaculture consumed in Serbia are marketed for human consumption as fresh or frozen. However, smoked products have seen a considerable surge in popularity. Smoking is one of most acceptable fish-processing method, because it does not require expensive equipment, the production period is short, acceptability on the Serbian market is very good, considering that the local population is accustomed to eat mostly smoked pork (*Kilibarda et al., 2009*). Modern consumers demand high quality food that retains the sensory characteristics and nutritive value of the raw material from which it is produced; also, it is expected to satisfy very demanding safety standards. This requirement is largely met by packaging the products in vacuum or modified atmosphere. The efficiency of vacuum in extending the shelf life of fish depends on several factors, such as the fish species, fat content, initial microbial cell count and most importantly, the storage temperature (*Babić Milijašević, 2017*). The most common reason for spoilage of smoked fish products is microbial activity. Microbial growth and the creation of products of their metabolic activity can lead to undesirable odour and taste, and the appearance of discoloration (*Leroi et al., 2001*).

Cyprinid species (common carp, bighead carp, and grass carp) are most commonly bred in Serbia (*Statistical Yearbook, 2020*). The aim of this research was to monitor changes of selected sensory properties and instrumental colour parameters (L*, a*, b*) of vacuum-packaged cold-smoked common carp (*Cyprinus carpio*) and cold-smoked bighead carp (*Hypophthalmichthys nobilis*) fillets during storage at 3 ± 0.5 °C and to determine the shelf life of the products.

Materials and Methods

Sampling

Samples from eleven common carp and eleven bighead carp of 2.5 ± 0.3 kg and 2.7 ± 0.5 kg live weight, respectively, were obtained from a fishpond where a semi-intensive rearing system was used. Fish were processed at freshwater fish processing plant using a standard procedure (killing by electrocution, descaling, evisceration, and filleting). Two fillets from each carp were made, and each fillet was divided into 2 portions, i.e. a total of 4 portions were obtained from 1 fish. After primary treatment, fish portions were washed and soaked in brine containing 25 g/L NaCl for 24 h at 5 °C and then pressed, laid on the grid in chambers for 1 h

at 12 °C. Smoking was performed on an automated smokehouse for 8 h at the temperature of 28 °C.

The 42 portions of cold-smoked common carp as well as the 42 portions of cold-smoked bighead carp were vacuum-packaged using the machine Variovac (Variovac Primus, Zarrentin, Germany), and a polyethylene-polyamide film (Suomen Union Verpackungs, Helsinki, Finland) with an oxygen permeability of 29–45 ml O_2 /m_2/24 h/atm (23 °C, 50% relative humidity, RH) and a water vapour permeability of 10–15 g/m2/24 h (38 °C, 90% RH) (1 atm = 101 325 Pa). All samples were stored under the same conditions, at the temperature of 3 ± 0.5 °C, and on days 1, 7, 10, 12, 14, 15, and 16 of storage, sensory tests and instrumental color determination were performed.

Sensory evaluation

The sensory evaluation was performed by six trained panelists. Panelists were trained according to international procedure (*EN ISO 8586, 2014*) and sensory assessment was carry out in test room who meet requirements of *EN ISO 8589, 2010*. Prior the sensory evaluation, samples were taken out from refrigerator to remain at room temperature for 30 min. For each day of examination, each assessor was provided with portion of fillet. The samples were evaluated for overall acceptability, with regard to odour, flesh colour, and texture using 1–5 intensity scale, with 5 corresponding to the most liked sample, and 1 corresponding to the least liked sample. The product was defined as unacceptable with a score of less than 2 points recorded by at least of 50% of the judges.

Instrumental colour determination

Colour of fish fillets was evaluated using colorimeter (Minolta Chroma Meter RC-400). The CIE system color profile of L*, a*, b* was measured by reflectance colorimeter using illuminant source D65, 8-mm aperture and 10° observation angle. The L* value represents lightness (L*= 0 for black, L*= 100 for white), while a* scale represents the red/green dimension, with positive values for red and negative ones for green. The b* scale represents the yellow/ blue dimension, with positive values for yellow and negative ones for blue (*CIE, 1976*). The colorimeter was calibrated throughout the experiment using a standard white ceramic tile (Y = 87.2; x = 0.3173; y = 0.3348). At each day of examination three measurement was carried out on surface of two portion.

Statistical analysis

The mean values and standard deviations were calculated by using column statistics with the processing of 6 values for each analyzed group. Significant

differences between groups were calculated using one-way ANOVA analysis by Tukey comparative test, Pearson's correlation analyses were performed on means of the values in the program Microsoft Office Excel (2010). Differences were evaluated as significant at $P < 0.05$.

Results and Discussion

The results of the sensory evaluation of cold-smoked common carp fillets and cold-smoked bighead carp fillets are presented in Table 1 (mean value ± standard deviation).

Table 1. Sensory evaluation of cold-smoked common carp fillets and cold-smoked bighead carp fillets stored under vacuum conditions at +3 ± 0.5°C.

Sensory parameter	Group	Storage time (days)						
		1	7	10	12	14	15	16
Odour	I	4.25±0.30^{a}	3.75±0.40^{b}	3.5±0.30^{b}	3.5±0.00^{b}	3.3±0.20^{b}	2.6±0.40^{c}	1.2±0.20^{d}
	II	5.0±0.00^{a}	5.0±0.00^{a}	4.8±0.25^{a}	4.0±0.40^{b}	3.8±0.50^{b}	3.5±0.40^{b}	2.8±0.50^{c}
Flesh texture	I	4.1±0.20^{a}	4.0±0.00^{a}	4.0±0.00^{a}	3.7±0.20^{a}	3.6±0.40^{a}	2.7±0.40^{b}	2.2±0.40^{b}
	II	5.0±0.00^{a}	5.0±0.00^{a}	4.8±0.25^{a}	4.6±0.50^{a}	4.4±0.40^{a}	3.7±0.50^{a}	3.1±0.20^{a}
Flesh colour	I	4.2±0.30^{a}	3.7±0.40^{b}	3.7±0.40^{b}	3.6±0.40^{b}	3.3±0.40^{b}	2.6±0.40^{c}	2.1±0.20^{d}
	II	5.0±0.00^{a}	5.0±0.00^{a}	4.8±0.25^{a}	4.1±0.60^{b}	4.1±0.60^{b}	3.6±0.50^{b}	3.4±0.60^{b}
Overall acceptability	I	4.2±0.30^{a}	3.7±0.40^{a}	3.5±0.40^{a}	3.6±0.20^{a}	3.5±0.40^{a}	2.6±0.40^{b}	1.2±0.20^{c}
	II	5.0±0.00^{a}	5.0±0.00^{a}	4.8±0.25^{a}	4.2±0.60^{a}	4.1±0.60^{a}	3.6±0.40^{a}	2.7±0.60^{b}

Group I: cold-smoked common carp samples; Group II: cold-smoked bighead carp samples; Same lowercase letters in a row indicate no significant differences ($p>0.05$)

At the beginning of storage period, colour, flesh texture, odour as well as overall acceptability were evaluated with very high scores in both group of samples. First day, color of common carp and bighead carp fillets was graded with high scores (4.2±0.30 and 5.0±0.00 respectively). Average grade of colour acceptability of cold smoked common carp and bighead carp fillets decreased during the storage. On the last day of experiment, reduced intensity of pink cream colouring of carp muscle was observed and it was evaluated as barely acceptable (2.1±0.20). The color of cold-smoked bighead carp muscle remained acceptable throughout the storage period (16. day it was evaluated as "acceptable" 3.4±0.60). In their research, *Kolodziejska et al. (2002)* and *Leroi et al. (2001)* found that colour intensity of vacuum packaged cold-smoked fish did not significantly change during three weeks of storage at 4°C. Likewise, *Bugueno et al. (2003)* did not reveal changes of smoked salmon colour packaged in vacuum and modified atmosphere during the storage at different temperature conditions. Numerous

studies in the European Union showed that the colour of smoked food products, specially smoked fish, is a main parameter that influence consumer decision to buy a particular type of food (*Espe et al., 2004*). *Johnston et al. (2000)* suggested that undesirable colour, as one of the most important cold-smoked salmon quality parameters, greatly reduces the cost of this product on the market.

In our research, textural changes was detected in common carp muscle during the storage. On day 16, softened texture and surface slime was discovered, and this sensory attribute were evaluated as "barely acceptable" (2.2±0.40). On the other hand, average scores of flesh texture of bighead carp muscle during the storage remained essentially unchanged. It was estimated as "very acceptable" at the beginning (5.0±0.00) and "acceptable" (3.1±0.20) at the end of storage period. The texture of cold-smoked fish has great importance for the quality of fish meat (*Lakshmanan et al., 2005*). During the storage, autolytic processes can cause changes in fish meat structure and consequently undesirable fish softening, even when the microbiota which usually causes spoilage is still not sufficiently developed to do so (*Olafsdottir et al., 2005; Dondero et al., 2004*).

During the experiment it was observed decrease of average grade of odour acceptability of cold smoked common carp fillets. On day 16 "musty" odour caused that odour score of common carp samples was below the acceptability limit of 2 (1.2±0.20). However, average scores of odor of bighead carp muscle during the storage period remained within the acceptability limit. It was estimated as "excellent" at the beginning (5.0±0.00) and "acceptable" (2.8±0.50) at the end of experiment. The high average odour score of bighead carp muscle in the present study had a positive impact on the overall acceptability of these fillets. *Truelstrup Hansen and Huss (1998)* and *Leroi et al. (1998)* in their examination of vacuum packaged cold-smoked trout, also found that during storage, the odour and taste of smoke intensity decreased, and became milder or almost neutral. Volatile substances that are usually produced by bacteria are the cause of undesirable odours in smoked fish (*Olafsdottir et al., 2005; Dondero et al., 2004*). These substances include trimethylamine, the volatile sulphur compounds, aldehydes, ketones, esters, hypoxanthine, and other low molecular weight substances. Trimethylamine is responsible for the typical "fishy" odour, indicator of spoilage.

As the results show, all estimated sensory characteristics of common carp samples received significantly lower ($P < 0.05$) score on day 15. A decrease of scores of the sensory attributes of cold-smoked bighead carp fillets was observed throughout the storage period. However, all estimated sensory characteristics were within the acceptability level.

Changes in fish meat begin at the moment fish dies, or already at the time of the catch and are the result of the activities of their own enzymes, the metabolism of microorganisms and the oxidation of lipids. Changes in the sensory characteristics of the fish usually result from the microbiological development. The decomposition of food ingredients and the growth of microorganisms cause an

unpleasant smell and taste as well as the production of visible pigmented or unpigmented colonies. The synthesis of polysaccharide extracellular materials and diffuse pigments results in sensory changes in the form of mucus formation and discoloration (*Fletcher et al., 2002*). On the other hand, chemical changes such as auto oxidation or enzymatic hydrolysis of fats may cause the rise of unpleasant smell and taste or, in the latter case, the activity of tissue enzymes may lead to unacceptable softening of the fish meat.

Changes in instrumental colour parameters of cold-smoked common carp fillets and cold-smoked bighead carp fillets are presented in Table 2. (mean value ± standard deviation).

Table 2. Instrumental determination of colour of cold-smoked common carp and cold-smoked bighead carp filets during the storage, CIE Lab system

	Group	Storage time (days)						
		1	7	10	12	14	15	16
L*	I	42.35±1.60^{a}	44.40±1.10^{a}	44.34±0.90^{a}	44.11±1.7^{a}	50.05±1.10^{b}	49.63±2.60^{b}	57.88±1.50^{c}
	II	50.26±0.20^{a}	49.10±1.90^{a}	50.80±0.25^{a}	52.15±3.3^{ba}	53.80±1.80^{a}	56.23±1.80^{b}	59.55±1.10^{c}
a*	I	4.70±0.47	4.14±0.30	4.67±0.28	3.94±0.50	4.60±2.32	4.00±0.38	4.52±0.45
	II	1,29±0.25	0.93±0.72	2.24±0.91	1.25±0.60	1.67±0.26	1.42±0.35	1.95±0.29
b*	I	5.92±0.71	7.01±0.25	5.54±2.27	7.18±1.94	7.54±1.87	5.93±0.54	6.76±1.08
	II	1.88±0.27	1.63±0.30	0.89±1.23	0.72±1.06	1.31±1.68	1.28±1.41	0.43±0.67

Group I : cold-smoked common carp samples; Group II: cold-smoked bighead carp samples; Same lowercase letters in a row indicate no significant differences ($p>0.05$)

In our research, from 1. to 12. day of experiment lightness (L*) of common carp samples did not change significantly ($P > 0.05$). After that period of time to the end of experiment lightness (L*) increased significantly ($P < 0.05$) by 24%. Lightness (L*) of bighead carp muscle was quite stable during the first fourteen days of storage. From that on L* value contineusly increase by the end of storage by 14.70%.Value for redness (a*) and yellowness (b*) remaid quit stable during the storage in both group of samples. Our results are in agreement with *Choubert et al. (2005)* who reported increase in L* value of vacuum packaged sliced smoked rainbow trout stored at chilled temperature. They concluded that during storage slices became paler, while changes in a* and b* parameters was not significant. During 40 days of storage at 4 °C *Rizo et al. (2015)* detected significantly increase of L*, a*, b* values of smoke-flavoured salmon packaged in water vapour permeable bags. The increase in lightness is explained by the water loss produced during storage which lead to greater water deposits on the fish surface. On the other hand, *Bugueno et al. (2003)* did not find any significant differences in L*, a*, b* values of smoked salmon packaged under vacuum and in modified atmosphere as a function on storage time. They suggested there was good colour stability of the examined product.

Table 3. Coefficients of correlation (r) of meat color (L*, a*, b*) and sensory attributes of cold-smoked common carp and cold-smoked bighead carp fillets

	Group	Odour	Flesh colour	Flesh texture	Overall acceptability
L*	I	-0.34	-0.54	-0.49	-0.51
	II	-0.37	-0.52	-0.40	-0.47
a*	I	0.25	0.15	0.09	0.21
	II	0.053	-0.023	0.120	0.007
b*	I	0.21	0.20	-0.25	-0.32
	II	0.27	0.24	0.26	0.25
Overall acceptability	I	0.98	0.91	0.87	-
	II	0.96	0.93	0.93	-

Group I : cold-smoked common carp samples; Group II: cold-smoked bighead carp samples

In our research moderate negative correlation between lightness (L*) and flesh colour as well as flesh texture and overall acceptability of cold-smoked common carp and cold-smoked bighead carp fillets was detected. Weak negative correlation between lightness (L*) and odour of cold-smoked common carp and bighead carp samples was determined.

Strong positive correlation between overall acceptability and odour, flesh colour and flesh texture of cold-smoked common carp and cold-smoked bighead carp samples was recorded.

Conclusion

Based on the sensory results, it was concluded that vacuum-packaged cold-smoked common carp samples remained acceptable for up to 15 days of storage, whereas vacuum packaged cold-smoked bighead carp samples remained unchanged until the end of the experiment.

Promena senzorskih svojstava ohlađenih hladno dimljenih fileta šarana (*Cyprinus carpio*) i hladno dimljenih fileta tolstolobika (*Hypophthalmichthys nobilis*) pakovanih u vakuumu

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Rezime

Cilj ovog istraživanja bio je da se ispitaju promene senzorskih svojstava i instrumentalnih vrednosti boje (L*, a*, b*) hladno dimljenih fileta šarana (*Cyprinus carpio*) i hladno dimljenih fileta tolstolobika (*Hypophthalmichthys nobilis*) pakovanih u vakuum koji su čuvani na temperaturi od 3 ± 0.5 °C, kao i da se odredi održivost proizvoda. Senzorska analiza i instrumentalno određivanje boje rađeni su 1, 7, 10, 12, 14, 15 i 16 dana. Petnaestog dana eksperimenta, sva ispitivana senzorska svojstva hladno dimljenih fileta šarana ocenjena su statistički značajno nižim ocenama ($P < 0.05$). Poslednjeg dana eksperimenta ustanovljen je užegao miris koji je ocenjen kao neprihvatljiv, dok je boja fileta šarana bila slabije izražena i bila je na granici prihvatljivosti. Tekstura mesa šarana bila je meka, a na površini fileta je utvrđeno prisustvo sluzi. Iako je ustanovljen pad vrednosti ocena senzorskih svojstava dimljenih fileta tolstolobika, sva senzorska svojstva su bila prihvatljiva tokom eksperimenta. Značajno povećanje L* vrednosti ($p < 0.05$) tokom ispitivanja utvrđeno je kod obe grupe uzoraka, dok su vrednosti za udeo crvene (a*) i žute (b*) boje bile stabilne. Na osnovu ispitanih senzorskih svojstava može se zaključiti da su hladno dimljeni fileti šarana pakovani u vakuumu bili prihvatljivi 15 dana, dok su hladno dimljeni fileti tolstolobika pakovani u vakuumu bili prihvatljivi do kraja ispitivanja.

Ključne reči: hladno dimljena riba, opšta prihvatljivost, rok trajanja, instrumentalni parametri boje

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CONTENTS OF SODIUM-CHLORIDE IN VARIOUS GROUPS OF LOCALLY MANUFACTURED MEAT

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Abstract: Sodium chloride (NaCl) is one of the most important food additives and it has a significant impact on the sensory and microbiological properties of meat products. According to the Regulation on the quality of ground meat, meat preparations and meat products (*Official Gazette of RS 50/2019*), the salt content in meat products is not defined. The average NaCl values in these products can be concluded by comparison with available experimental and literature data. The aim of this study was to examine the content of sodium chloride in different meat products from 3 different production batches locally produced. A total of 42 samples were tested: Kulen and Čajna sausage (fermented sausages), dry tenderloin (cured meat products), smoked tenderloin (smoked products), hot dog (finely chopped boiled sausage), Serbian sausage (coarsely chopped boiled sausage) and pancetta (bacon). The highest average sodium chloride content was found in dry tenderloin (4.49g/100 g) while the lowest content was measured in hot dogs (1.88g/100 g). Comparing the obtained values of sodium chloride content with the values obtained by other authors for fermented products (Kulen and Čajna sausage), the tested products had significantly higher values of salt content, while the lowest average content of sodium chloride was found in smoked tenderloin samples. For other products, the content of the tested parameter was similar to the values reported in the literature. After the analysis of available samples, it was determined that the manufacturer adhered to the prescribed amounts of NaCl, according to the recipe, in every product. There weren't any notable deviations in the preparation of monitored meat products.

Key words: sodium chloride, meat products

Introduction

Sodium chloride (NaCl) is one of the most commonly used additives in the meat industry because of its low cost and its functionality (*Ruusunen and Puolanne, 2005*). Cured meat products exist since ancient times as a result of the need to preserve meat for a longer period of time. Salting and drying prolong the shelf life of this perishable food. This process also provides microbiological stability and improves organoleptic properties such as texture.It is , additionally, used for flavouring, as a flavour enhancer, and is also responsible for water binding capacity and giving desired textural properties to processed meat. Cured meat products are foods highly valued by consumers as they are one of the most consumed groceries in people's nutrition. The properties that make these food stuffs particularly appealing are the result of the transformation of proteins and lipids that give these products their characteristic aroma and taste (*Balestra and Petracci, 2019; Domínguez et al., 2017*). In addition, the salinity caused by sodium chloride improves the perception of meat taste, which is an significant factor in the overall acceptability of meat products. Therefore, the reduction of sodium in processed meat can negatively affect the overall quality of the final product (*Ruusunen and Puolanne, 2005; Pietrasik and Gaudette, 2015).*

Sodium chloride is an excellent preservative, which inhibits the growth and development of unwanted microorganisms, prevents rapid spoilage, and increases the shelf life of cured meat products (*Inguglia et al., 2017*). Exposure to NaCl causes osmotic shock to microorganisms by binding water molecules with a consequent decrease in water activity (a_w) below optimal growth conditions, resulting in water loss from the cell causing microbial cell death or slowing their growth (*Tailor and Davidson, 2007; Yotsuyanagi et al., 2016*). However, salt accelerates the oxidation of lipids and consequently generates unwanted changes in the colour and taste of meat and meat products, reducing their shelf life. In some cases, lipid oxidation is desirable, such as the development of the typical aroma of some meat products such as ham and sausages (*Mariutti and Bragagnolo, 2017*).

One of the main functions of salt in processed meat is the solubilization of functional myofibrillar proteins and improving water-holding capacity of meat products. As the salt penetrates the meat, the osmotic pressure around the muscle cells becomes higher than the one inside the cells, which consequently leads to the so-called process of so called osmotic dehydration. This process leads to an increase in the ability of proteins to bind water, resulting in a change or improvement in the texture of meat products. Increasing the water-binding capacity of meat reduces water loss during heat treatment, which improves the softness and juiciness of meat products *(Desmond, 2006; Domínguez et al., 2017; Morales et al., 2013).*

Meat and meat products are one of the components of the diet that contribute the most to sodium intake in the diet, with approximately 18-21% of daily sodium intake. The sodium content of meat products shows large variations in the degree of meat processing (fresh, dried, and processed meat), with unprocessed meat containing less than 0.1 g of sodium per 100 g of meat (*Aaslyng et al., 2014; De Marchi et. al., 2017).*

The results of the DASH study (Dietary Approaches to Stopping Hypertension) show a linear correlation between salt intake and blood pressure. The link between excessive sodium intake and the development of hypertension has prompted public health and regulatory authorities to issue recommendations to reduce dietary salt intake (*Desmond et al., 2019*). *Kloss et al. (2015),* according to the data of the European Commission, state that the countries of Eastern and Southern Europe show the highest rates of salt consumption. According to these data, salt consumption in adults in most European countries ranges from 7 to 13 g per day. Germany, Cyprus, Bulgaria and Latvia report the lowest salt intake (6.3 - 7.3 g/day), while the Czech Republic, Slovenia, Hungary and Portugal report the highest salt intake (12.3 - 13.6 g/day). *Powles et al. (2013)* report significantly different levels of salt intake with the lowest intake values observed in Denmark, the Netherlands and Belgium (8.3 - 8.8 g/day), and the highest in Hungary, Slovenia, Slovakia, Portugal and Italy (10.7 - 11, 2 g/day).

With increasing economic, health and consumer awareness, countries such as Finland, the UK, the EU, the US, and many other countries have formed national strategies to reduce salt consumption (*Aaslyng et al., 2014*). According to data gathered from previous research on sodium intake, populations from around the world are consuming much more sodium than is physiologically necessary. The current recommendations of the World Health Organization (*WHO, 2012*) are 5 g/day salt of salt, but there is a tendency to further reduce sodium intake to <2 g/day sodium (less than 5 g/day salt) in adults (strong recommendation), with a new goal of reducing dietary sodium intake by 30% before 2025. Changing consumer lifestyles and the easy availability of highly processed and fast foods have led to increased salt consumption. There is currently a great deal of consumer concern regarding salt intake and its prevalence in the diet worldwide. However, even with the development of modern canning practices, NaCl is still necessary for processed meat products. For the industry to actively involve in the salt reduction process, it is essential that products must be acceptable in terms of all quality parameters: shelf life, food safety, product texture, production yield, taste, and consumer acceptability throughout the shelf life (*Aaslyng et al., 2014*).

Although great progress has been made in the development of ingredients to replace salt and flavour enhancers in recent decades, there is a persisting problem of negative sensory effects that correlates with the use of these substances. The challenges that remain are the result of a need to use other ionic compounds to replace the functions of water retention, protein binding, and fat binding in foods in

which sodium chloride has been reduced while maintaining adequate microbiological safety (*Balestra and Petracci, 2019*). The author *Lilić (2016)* shows in his research that the reduction of sodium chloride content by replacement with potassium chloride and ammonium chloride has no significant effect on the sensory characteristics and colour of dried meat if these substances are added in an appropriate ratio.

Traditional practice in small meat processing plants leads to great variability in product properties (heterogeneous quality) because there is no strict uniformity in production. As sensory characteristics are one of the most important components of the quality of cured meat products, it is important to create a product with such attributes that would be attractive to the consumer, but it is also important to ensure continuous product quality, i.e. low variability of product characteristics. However, so far little has been done in the field of assessing the repeatability of the quality of traditionally cured meat products manufactured at low-capacity plants (*Jokanović et al., 2020*). On the other hand, the study conducted by *Rason et al. (2006)* shows that the internal composition of traditional dry sausages from 6 smaller production plants was homogeneous despite the apparent heterogeneous matrix.

The aim of the study was to examine the content of sodium chloride in meat products originating from 3 different production batches produced by a local manufacturer in order to gain insight into the uniformity of product quality and compliance with the manufacturer's specification.

Material and Methods

Contents of NaCl were determined from the meat products that were sampled from a local producer from a small-capacity production plant. Five groups of meat products were examined, as follows: fermented dry sausages (Kulen and Čajna sausage), cured meat products (dry tenderloin), smoked products (smoked tenderloin), cooked products (hot dogs and Serbian sausage), and bacon (pancetta). Each product (originating from 3 different batches) was tested in duplicate, to determine how standardized the salting process was.

The NaCl content was determined volumetrically, by the Volhard method (SRPS ISO 1841-1: 1999).

The results of our research were statistically processed (Statsoft Inc. Statistics for Windows, Version 5.0.) and presented in tables as the arithmetic mean ($\bar{X}$), the standard error of the arithmetic mean ($S\bar{X}$), the standard deviation (SD), the variation interval (minimum – maximum) and coefficient of variation (CV).

Results and Discussion

The role of salt in meat products is multiple (inhibition of microorganism growth, ability to bind water, taste enhancer…). According to the Regulation on the quality of ground meat, meat preparations, and meat products (*Official Gazette of RS 50/2019*), the salt content in meat products is not defined, even though sodium chloride is one of the essential ingredients of dry fermented products. Based on experimental and literature data, the amount and method of adding table salt are specific to each group of meat products (*Prica et al., 2013*).

Table 1. shows the contents of sodium chloride in various meat products displayed as a percentage (%). Table 2. shows the average sodium chloride content in all 6 measurements (two measurements, three production batches).

The lowest coefficient of variation (CV), shown in Table 2, was recorded for following products: smoked tenderloin (2.92%) and fermented sausages - Čajna sausage (3.91%) and Kulen (4.87%). Such a low CV (CV <5%) may be the result of a relatively small number of samples (a total of 6 samples per product). The highest coefficient of variation was recorded in the samples of hot dogs (8.70%), pancetta (8.69%) and dry tenderloin (8.34%). Low coefficients of variation (CV <10%) indicate that, although there were variations in the preparation of meat products, they were not notable, i.e. the manufacturer adhered to the prescribed amount of NaCl added to each product, according to the recipe.

The average NaCl content in fermented sausages ranged from 3.98% to 4.49%. In a study by *Prica et al. (2013)* the average reported NaCl content in fermented sausages was 3.77%, while *Vuković et al. (2011)* state that the NaCl content in Kulen varied from 3.40 - 3.80%. According to the data reported by *Kurčubić et al. (2011),* the mean value of NaCl content in Kulen in the three tested production batches was 3.45 g/100 g. The data provided by *Pećanac et al. (2017)* shows the NaCl content in Čajna sausage of 3.98%. *Branković Lazić et al. (2019)* in their research state that the NaCl content in fermented sausages ranged from 2.81% to 3.37%, depending on the applied manufacturing process. All mentioned values of salt content in the literature were lower than the values obtained in our research.

Table 1. NaCl content (g/100g) in different meat products

Product group	Product	Production batch	NaCl content	$\bar{X}$	$S\bar{X}$	SD	Interval of variation	
							Min	Max
Fermented meat products	Kulen sausage	1	4.44	4.38	0.06	0.08	4.32	4.44
		1	4.32					
		2	4.00	3.99	0.01	0.01	3.98	4.00
		2	3.98					
		3	4.44	4.36	0.08	0.11	4.28	4.44
		3	4.28					
	Čajna sausage	1	4.19	4.14	0.05	0.07	4.09	4.19
		1	4.09					
		2	4.45	4.47	0.02	0.03	4.45	4.49
		2	4.49					
		3	4.22	4.34	0.12	0.16	4.22	4.45
		3	4.45					
Dry meat products	Dry tenderloin	1	4.95	4.92	0.04	0.05	4.88	4.95
		1	4.88					
		2	4.20	4.10	0.11	0.15	3.99	4.20
		2	3.99					
		3	4.49	4.46	0.03	0.04	4.43	4.49
		3	4.43					
Smoked meat products	Smoked tenderloin	1	3.00	2.98	0.02	0.03	2.96	3.00
		1	2.96					
		2	2.83	2.80	0.02	0.03	2.77	2.83
		2	2.77					
		3	2.87	2.89	0.01	0.02	2.87	2.90
		3	2.90					
Finely chopped boiled sausages	Hot dog	1	1.77	1.81	0.04	0.06	1.77	1.85
		1	1.85					
		2	2.12	2.08	0.05	0.06	2.03	2.12
		2	2.03					
		3	1.76	1.74	0.02	0.03	1.72	1.76
		3	1.72					
Coarsely chopped boiled sausages	Serbian sausage	1	2.54	2.56	0.02	0.03	2.54	2.58
		1	2.58					
		2	2.46	2.41	0.05	0.07	2.36	2.46
		2	2.36					
		3	2.78	2.77	0.01	0.01	2.76	2.78
		3	2.76					
Bacon	Pancetta	1	2.69	2.72	0.03	0.04	2.69	2.75
		1	2.75					
		2	2.82	2.84	0.02	0.02	2.82	2.85
		2	2.85					
		3	3.26	3.26	0.00	0.00	3.26	3.26
		3	3.26					

Table 2. Average NaCl content (g / 100g) in different meat products in all 6 measurements

Product	Production batch	$\bar{X}$	$S\bar{X}$	SD	Interval of variation		CV (%)
					Min	Max	
Kulen sausage	6	4.24	0.21	0.08	3.98	4.44	4.87
Čajna sausage	6	4.32	0.17	0.07	4.09	4.49	3.91
Dry tenderloin	6	4.49	0.37	0.15	3.99	4.95	8.34
Smoked tenderloin	6	2.89	0.08	0.03	2.77	3.00	2.92
Hot dog	6	1.88	0.16	0.07	1.72	2.12	8.70
Serbian sausage	6	2.58	0.17	0.07	2.36	2.78	6.41
Pancetta	6	2.94	0.26	0.10	2.69	3.26	8.69

The average NaCl content in dry tenderloin was 4.49% (Table 2). *Marchi et al. (2017)* report a value of 3.63% for NaCl content for this group of products. *Ganić et al. (2012)* examined the chemical composition of samples of high-quality tenderloin and tenderloin from industrial production, and obtained average values for NaCl content of 7.70% for tenderloin produced by artisanal production, or 4.96% for samples of origin from industrial production. The results obtained in the research of *Tomljanović (2015)* show that the share of salt in cured meat sausages was 4.39%. According to *Kurčubić et al. (2011),* the salt content in dry pork ham in the three tested production batches was constant, and the mean value of sodium chloride content was 5.72 g/100 g. In study by *Pleadin et al. (2015)* the salt content was analyzed in different meat product categories and the results differed from 6.34% (prosciutto) to 6.52% (dry ham). The NaCl content in smoked tenderloin (Table 1), displayed as a percentage. The NaCl content in this product ranged from 2.80% to 2.98% between batches, in contrast to the study by *Pleadin et al. (2015)* who reported a value of 5.34% for the same product. In their research, *Stamenković (2004)*, stated that before preparing meat for smoking process, amount of up to 3.76% of NaCl is added, which correlates with the obtained result of NaCl content in the finished product in the amount of 3.44%, which are slightly higher than those obtained in analyzed products.The results in our study show that the NaCl content in boiled meat products ranged from 1.95% (average value for hot dogs) to 2.50% (average value for Serbian sausage), as shown in Table 2. In the production of boiled sausages (finely chopped boiled sausages, coarsely chopped boiled sausages, boiled sausages with pieces of meat), kitchen salt is is usually added in the amount of 1.8% to 2.2% (this is considered the "normal salting") (*Vuković, 1998*). Average values for the same product group are 2.19%, according to research by *Aaslyng et al. (2014)*; values stated by *Peulić et al. (2019)* are 1.57 - 2.26 g/100g of product. Authors *Prica et al. (2013)* measured 3.06% sodium chloride on average in finely chopped boiled sausages from the Novi Sad market. Results of the study by *Đorđević et al. (2017)* conducted on Serbian sausage, sampled from 11 different producers from the territory of the Republic of Serbia, show the range of NaCl with a range of 1.60% to 2.50%. All mentioned studies indicate that the NaCl content in products that we tested was very similar to the experimental results

presented in the literature.The obtained NaCl content for dry bacon (bacon category) with a mean value of 2.94%, was lower than the one obtained in the study of *Guofeng et al., (2010),* with an average salt content of 5.07%, as well as the values obtained by *Pleadin et al. (2015),* who reported the salt content in bacon of 5.52% and semi-durable bacon of 5.09%, as well as in pancetta - 5.57% (durable bacon). Previous research by this author on two different pancetta samples shows an even higher salt content in the range of 8.56% - 9.08% (*Pleadin et al., 2013*) compared to our samples. On the other hand, *Marchi et al., (2017)* state a value of 2.66% for the NaCl content in this product group, which is slightly lower result compared to the values from our study.

Conclusion

Kitchen salt is one of the most important and widespread food additives that has not only a preservative effect, but also has a significant impact on the sensory and microbiological properties of meat products.

The highest average sodium chloride content was found in dry tenderloin (4.49 g/100 g) while the lowest content was measured in hot dogs (1.88 g/100 g). Comparing the obtained values of sodium chloride content with the values measured by other authors for fermented products (Kulen and Čajna sausage), the examined products had significantly higher values of salt concentration.The lowest content of sodium chloride was found in smoked tenderloin samples. For other products, the content of the tested parameter was similar to the values reported in the literature. The World Health Organization recommends that the daily intake of salt for adults, healthy people should not exceed 5 g/day. Since the obtained results indicate that in some tested samples the measured amount of sodium chloride was very close to the upper limit of the recommended value of daily salt intake, measured per 100 g of meat product, it is necessary to continuously and systematically control and reduce sodium chloride in meat products. Also, there is a persisting need to make data on the salt content available on the label of each of these products so it could be easier for consumers to make a decision when buying a product. Depending on the type of meat product, the NaCl quantity did not notably differ between batches. This indicates the identical application of the prescribed recipe of the producer and similarity of the method of processing meat products. We concluded that the salting technology is strictly followed in the production process.

Sadržaj natrijum-hlorida u različitim proizvodima od mesa lokalnog proizvođača

Tamara Stamenić, Maja Petričević, Ljiljana Samolovac, Slađana Šobajić, Bogdan Cekić, Marija Gogić, Vladimir Živković

Rezime

Natrijum hlorid (NaCl) predstavlja jedan od najvažnijih aditiva koji se može naći u hrani i ima značajan uticaj na senzorna i mikrobiološka svojstva proizvoda od mesa. Prema pravilniku o kvalitetu usitnjenog mesa, poluproizvoda od mesa i proizvoda od mesa (Sl. glasnik RS 50/2019), sadržaj soli u proizvodima od mesa nije definisan, te se prosečne vrednosti NaCl u ovim proizvodima mogu zaključiti komparacijom sa dostupnim eksperimentalnim i literaturnim podacima. Cilj ovog istraživanja je bio da se ispita sadržaj natrijum-hlorida u različitim proizvodima od mesa iz 3 različite proizvodne šarže jednog lokalnog proizvođača. Ukupno je ispitano 42 uzorka, i to: kulen i čajna kobasica (fermentisane kobasice), suva (fino usitnjena barena kobasica), srpska kobasica (grubo usitnjena barena kobasica) i pančeta (slanina). Najveći prosečan sadržaj natrijum-hlorida utvrđen je u suvoj pečenici (4.49 g / 100 g) dok je najmanji sadržaj izmeren u viršli (1.88 g/100 g). Upoređivanjem dobijenih vrednosti sadržaja natrijum-hlorida sa vrednostima koje su drugi autori dobili za fermentisane proizvode (kulen i čajna kobasica), ispitivani proizvodi imali su znatno više vrednosti koncentracije soli, dok je najmanji prosečni sadržaj natrijum-hlorida utvrđen u uzorcima dimljene pečenice. Za ostale proizvode sadržaj ispitivanog parametra je bio sličan vrednostima navedenim u literaturi. Nakon izvršenih analiza dostupnih uzoraka utvrđeno je da se proizvođač pridržavao propisanih količina NaCl koji se dodaje, prema recepturi, u svaki od proizvoda, te da nije bilo značajnih odstupanja prilikom pripreme praćenih proizvoda od mesa.

Ključne reči: natrijum-hlorid, proizvodi od mesa

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Corresponding author: Milan M.Petrović, **e-mail address**
Review paper

Example 2

EFFECTS OF REARING SYSTEM AND BODY WEIGHT OF REDBRO BROILERS ON THE FREQUENCY AND SEVERITY OF FOOTPAD DERMATITIS

Zdenka Škrbić, Zlatica Pavlovski, Miloš Lukić, Veselin Petričević

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